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C 1830121

What Is Claimed Is:

1. Computer readable medium having recorded thereon the nucleotide sequence depicted in SEQ ID NO:1, a representative fragment thereof or a nucleotide sequence at least 99.9% identical to the nucleotide sequence depicted in SEQ ID NO:1.
2. Computer readable medium having recorded thereon any one of the fragments of SEQ ID NO:1 depicted in Table 1a or a degenerate variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.
3. The computer readable medium of claim 1, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.
4. The computer readable medium of claim 3, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.
5. A computer-based system for identifying fragments of the *Haemophilus* genome of commercial importance comprising the following elements;
- a) a data storage means comprising the nucleotide sequence of SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1;
 - b) search means for comparing a target sequence to the nucleotide sequence of the data storage means of step (a) to identify homologous sequence(s), and
 - c) retrieval means for obtaining said homologous sequence(s) of step (b).

6. A method for identifying commercially important nucleic acid fragments of the *Haemophilus* genome comprising the step of comparing a database comprising the nucleotide sequence depicted in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence is not randomly selected.

7. A method for identifying an expression modulating fragment of *Haemophilus* genome comprising the step of comparing a database comprising the nucleotide sequence depicted in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence comprises sequences known to regulate gene expression.

8. An isolated protein-encoding nucleic acid fragment of the *Haemophilus influenzae* Rd genome, wherein said fragment consists of the nucleotide sequence of any one of the fragments of SEQ ID NO:1 depicted in Table 1a or a degenerate variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.

9. A vector comprising any one of the fragments of the *Haemophilus influenzae* Rd genome depicted in Table 1a or a degenerate variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.

10. An isolated fragment of the *Haemophilus influenzae* Rd genome, wherein said fragment modulates the expression of an operably linked

open reading frame, wherein said fragment consists of the nucleotide sequence from about 10 to 200 bases in length which is 5' to any one of the open reading frames depicted in Table 1a or a degenerate variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.

5 11. A vector comprising any one of the fragments of the *Haemophilus influenzae* Rd genome of claim 8.

12. An organism which has been altered to contain any one of the fragments of the *Haemophilus* genome of claim 8.

10 13. An organism which has been altered to contain any one of the fragments of the *Haemophilus* genome of claim 10.

15 14. A method for regulating the expression of a nucleic acid molecule comprising the step of covalently attaching 5' to said nucleic acid molecule a nucleic acid molecule consisting of the nucleotide sequence from about 10 to 100 bases 5' to any one of the fragments of the *Haemophilus* genome depicted in Table 1a or a degenerate variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.

20 15. An isolated nucleic acid molecule encoding a homolog of any one of the fragment of the *Haemophilus* genome depicted in Table 1a, excluding the fragments of SEQ ID NO:1 depicted in Table 1b wherein said nucleic acid molecule is produced by the steps of:

a) screening a genomic DNA library using any one of the fragments of the *Haemophilus* genome depicted in Table 1a as a target sequence;

25 b) identifying members of said library which contain sequences which hybridize to said target sequence;

c) isolating the nucleic acid molecules from said members identified in step (b).

5 16. An isolated DNA molecule encoding a homolog of any one of the fragments of the *Haemophilus* genome depicted in Table 1a, excluding the fragments of SEQ ID NO:1 depicted in Table 1b wherein said nucleic acid molecule is produced by the steps of:

- a) isolating mRNA, DNA, or cDNA produced from an organism;
- 10 b) amplifying nucleic acid molecules whose nucleotide sequence is homologous to amplification primers derived from said fragment of said *Haemophilus* genome to prime said amplification;
- c) isolating said amplified sequences produced in step (b).

15 17. An isolated polypeptide encoded by any one of the fragments of the *Haemophilus influenzae* Rd genome depicted in Table 1a or by a degenerate variant of said fragment, excluding the fragments of SEQ ID NO:1 depicted in Table 1b.

18. An isolated polynucleotide molecule encoding any one of the polypeptides of claim 17.

20 19. An antibody which selectively binds to any one of the polypeptides of claim 17.

20. A method for producing a polypeptide in a host cell comprising the steps of:

25 a) incubating a host containing a heterologous nucleic acid molecule whose nucleotide sequence consists of any one of the fragments of the *Haemophilus influenzae* Rd genome depicted in Table 1a or a degenerate

variant thereof, excluding the fragments of SEQ ID NO:1 depicted in Table 1b under conditions where said heterologous nucleic acid molecule is expressed to produce said protein, and

b) isolating said protein.

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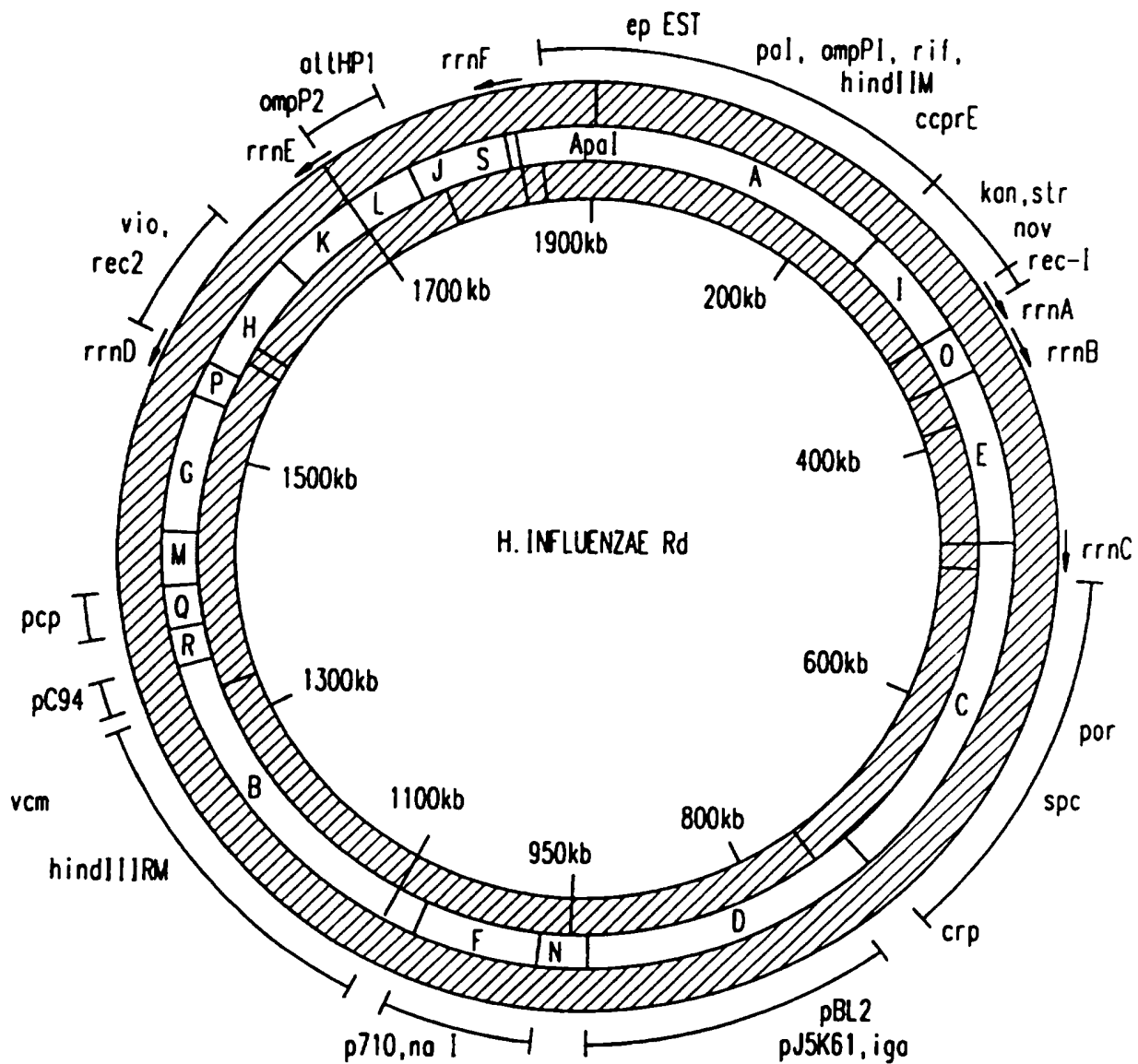


FIG.1

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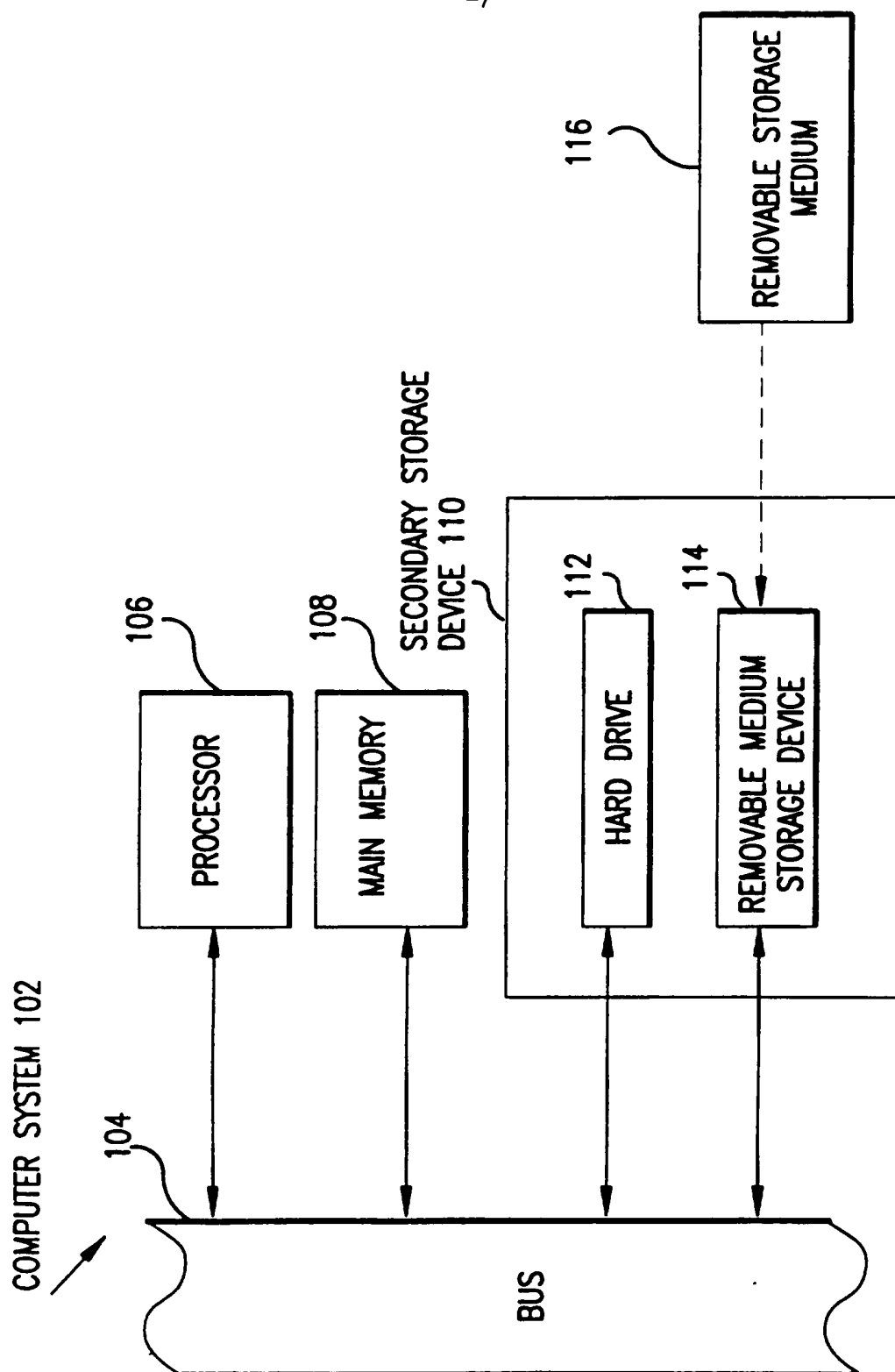


FIG.2

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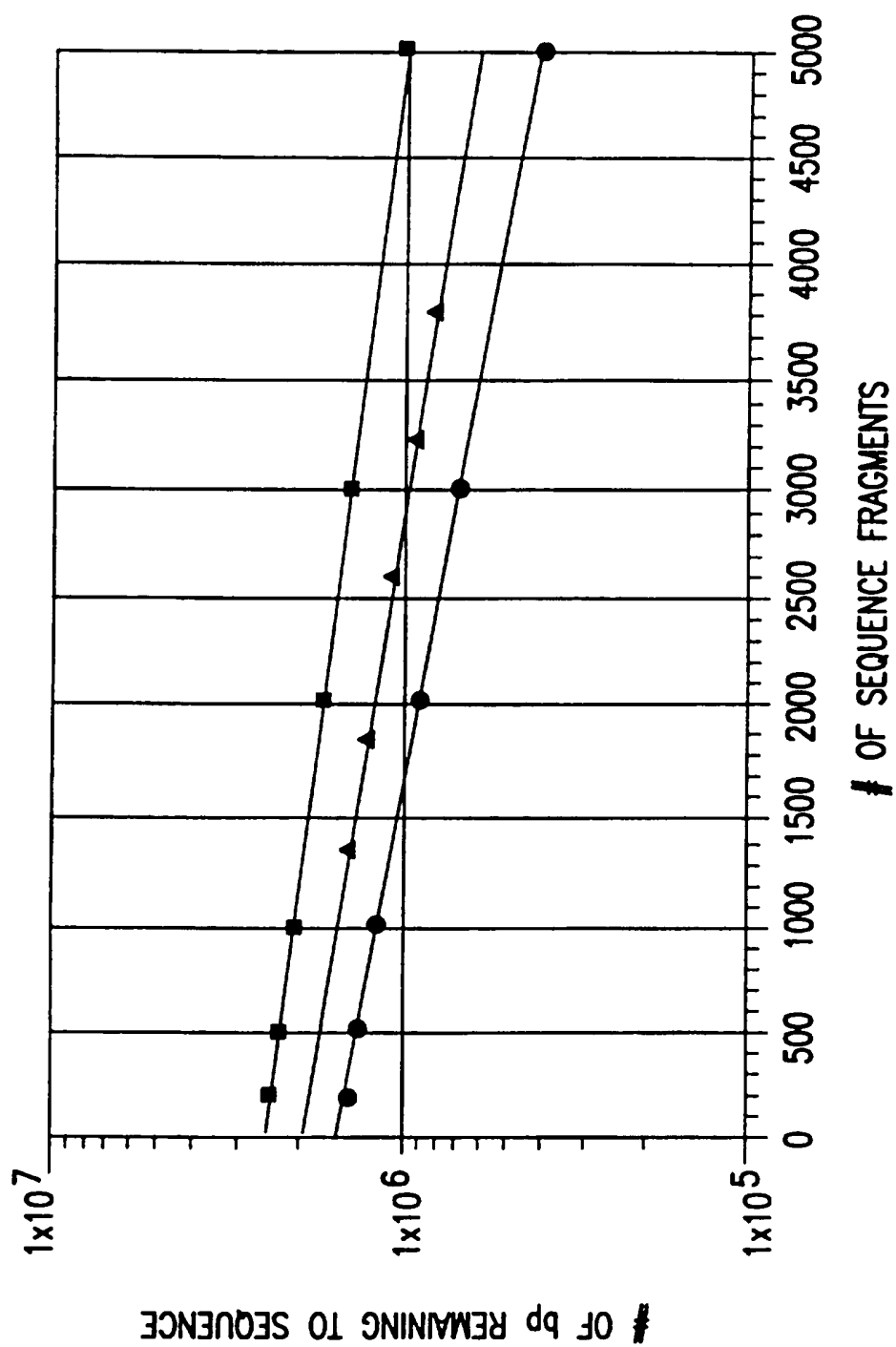


FIG. 3

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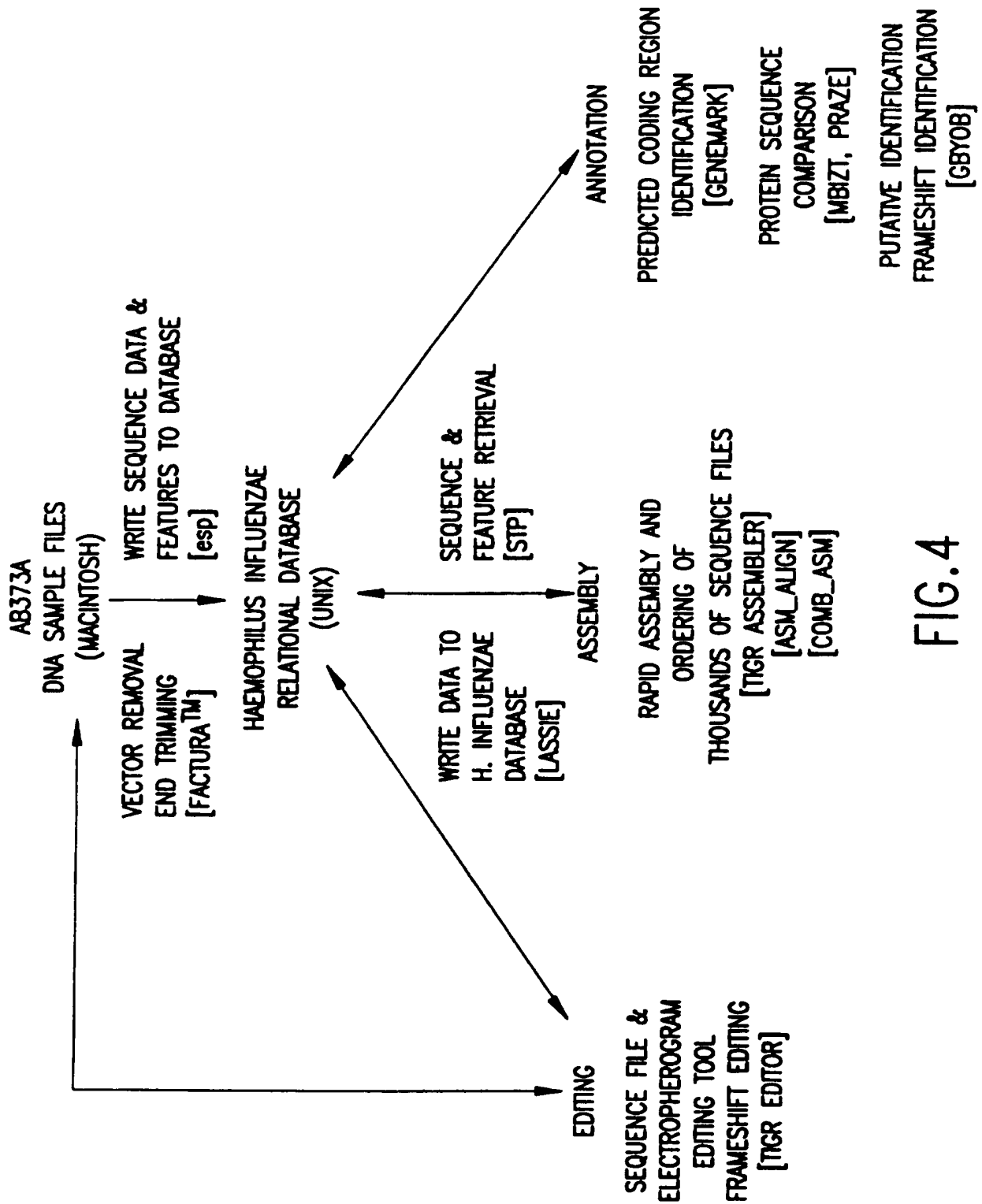
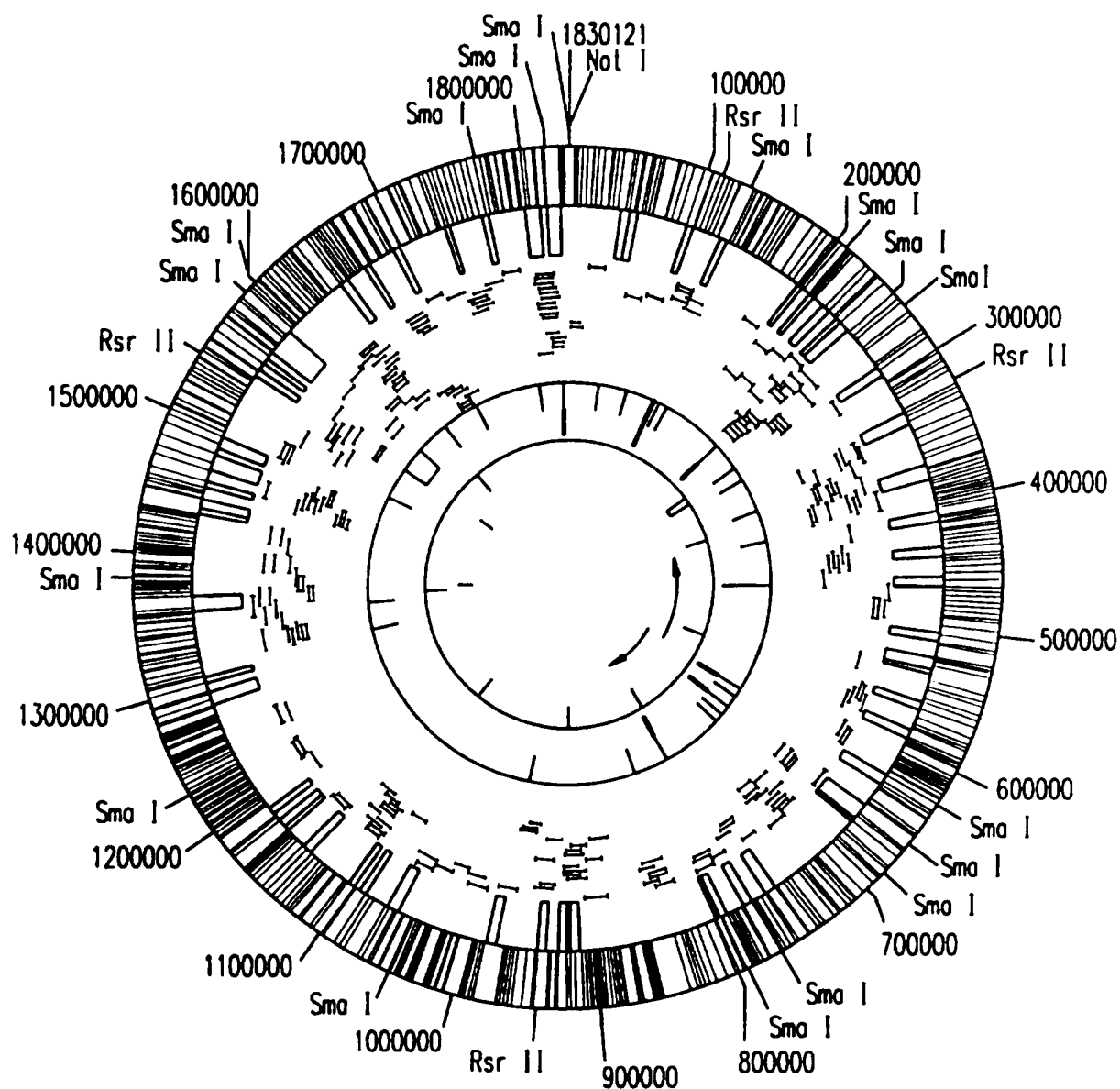


FIG.4

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HAEMOPHILUS INFLUENZAE

FIG.5

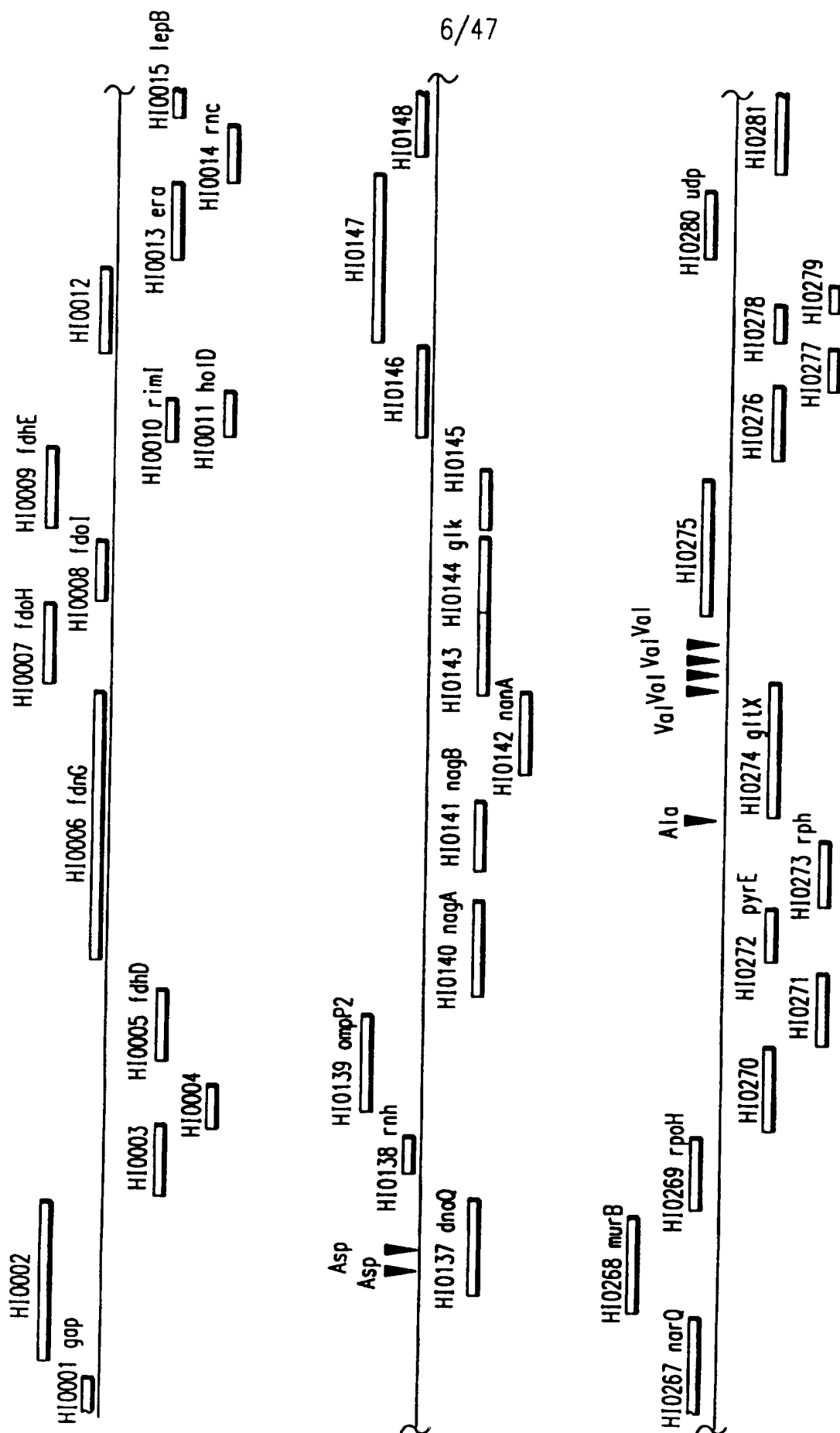


FIG. 6A

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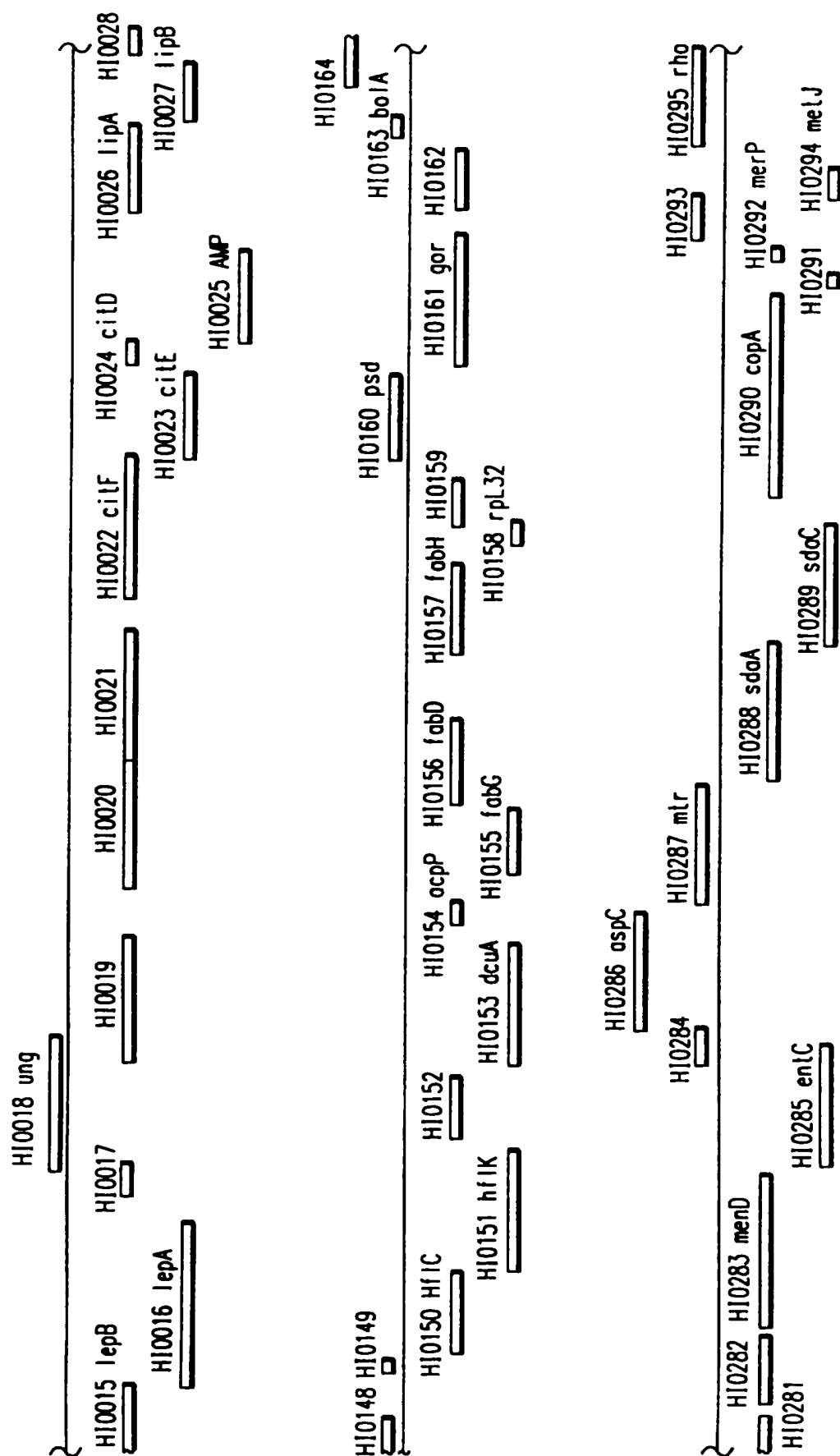


FIG. 6B

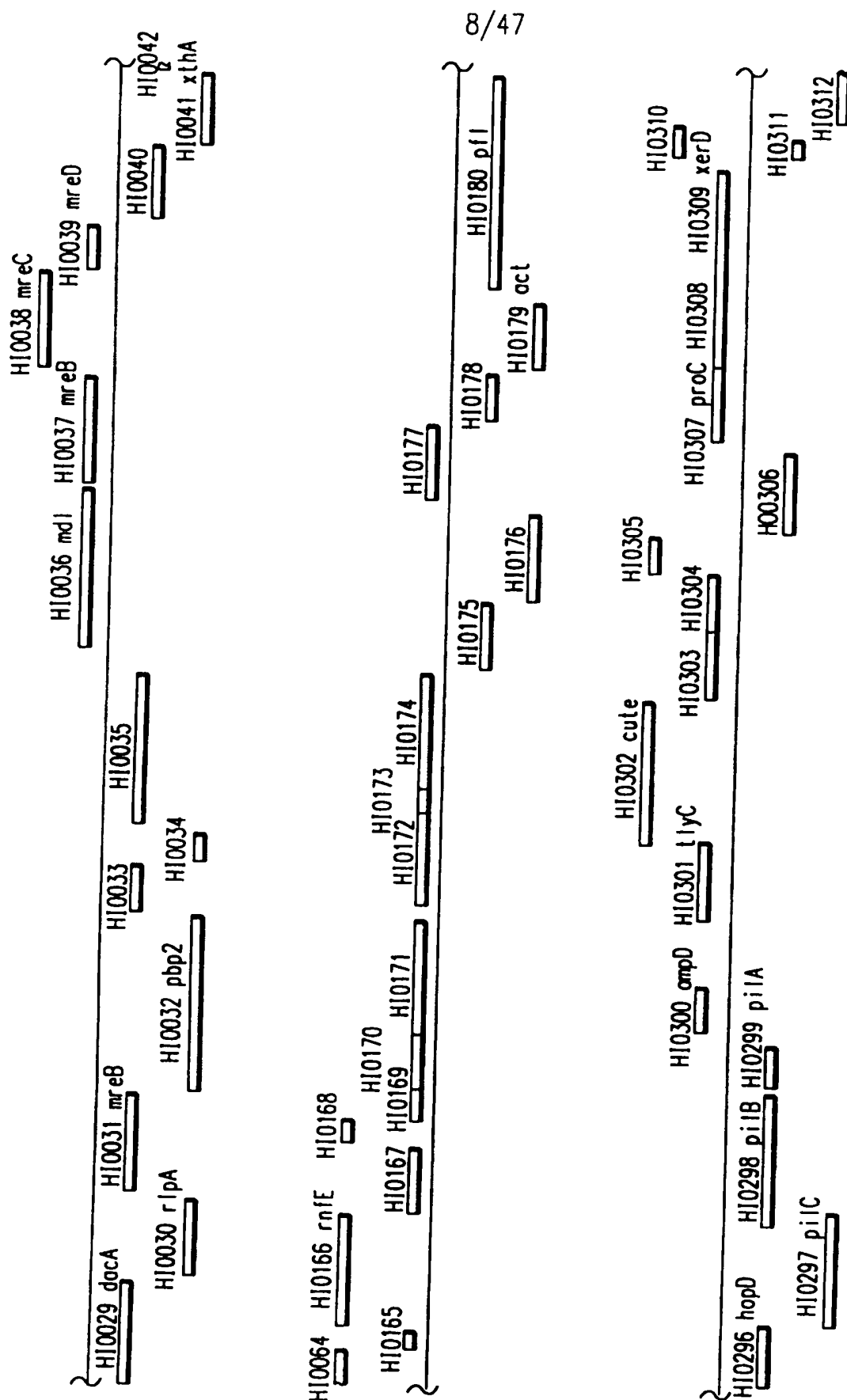


FIG.6C

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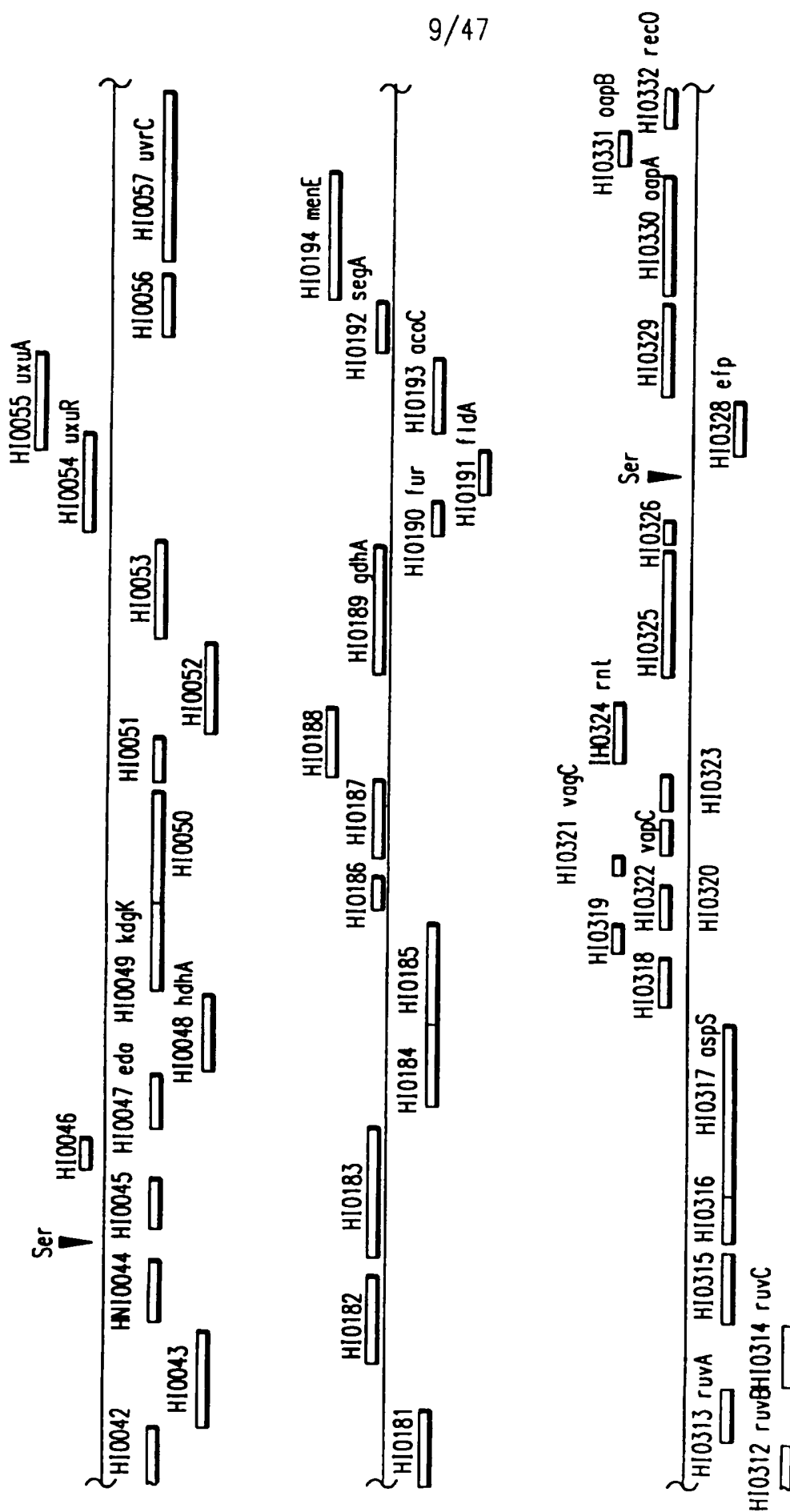


FIG. 6D

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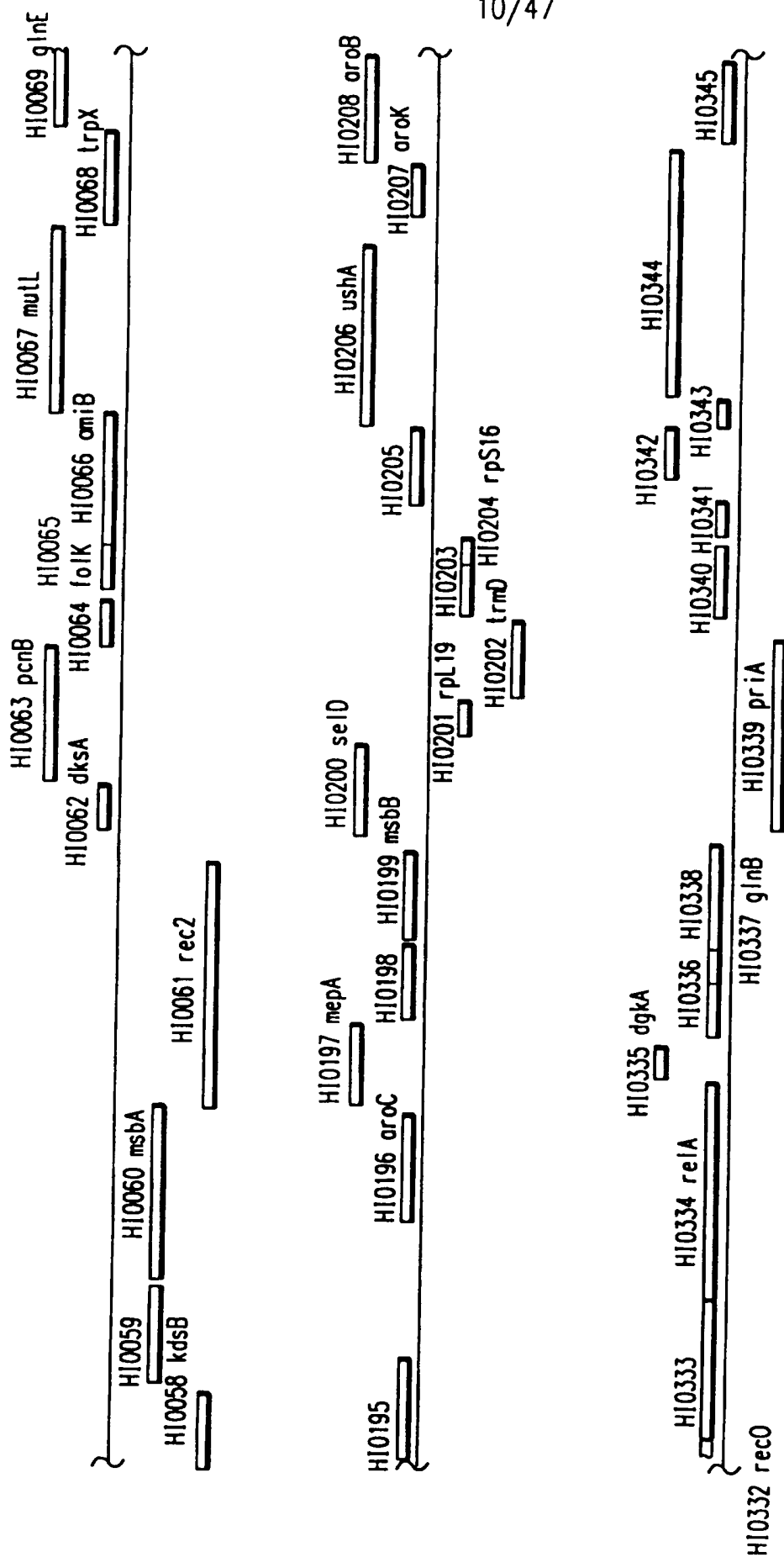


FIG.6E

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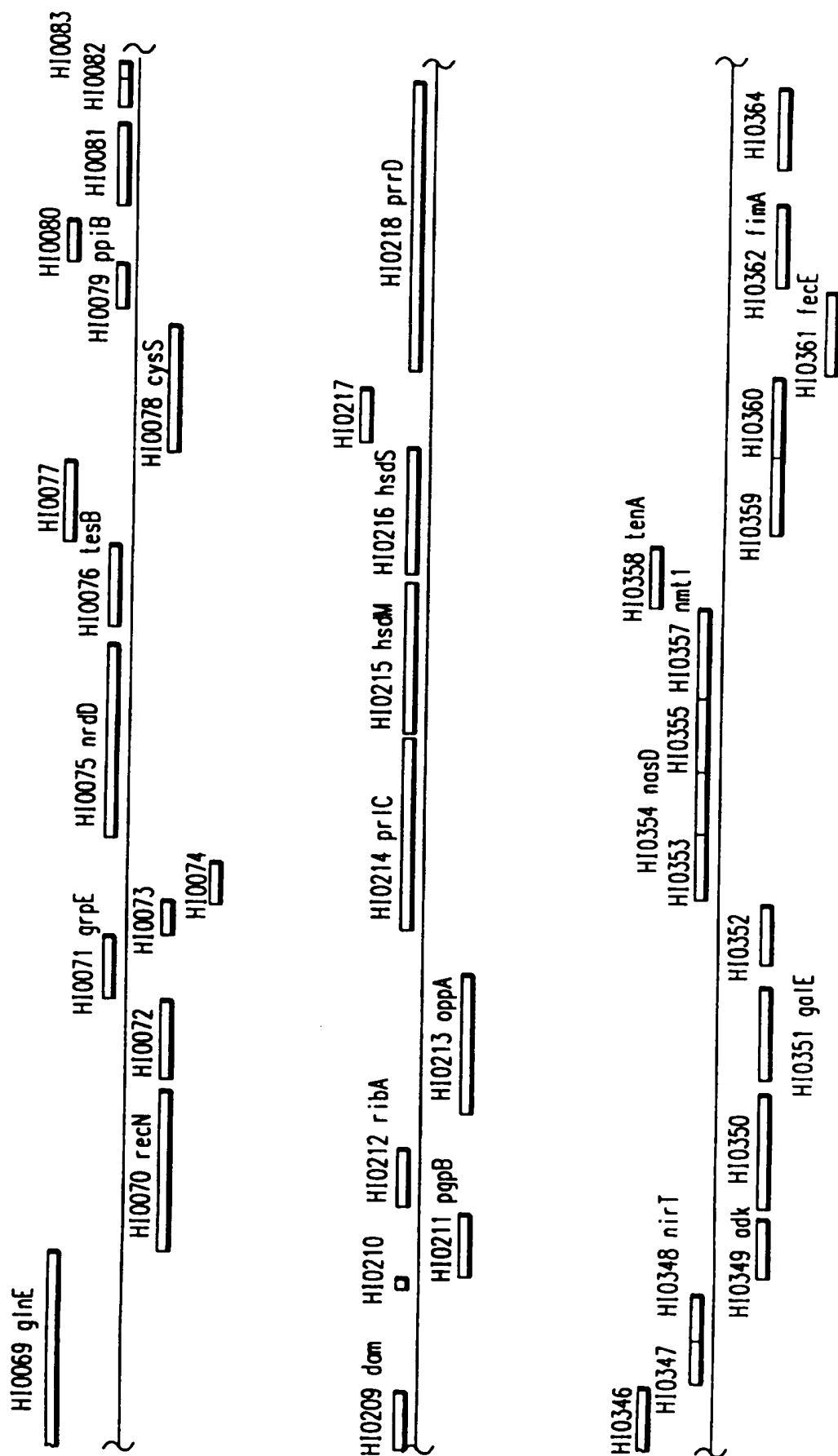


FIG. 6F

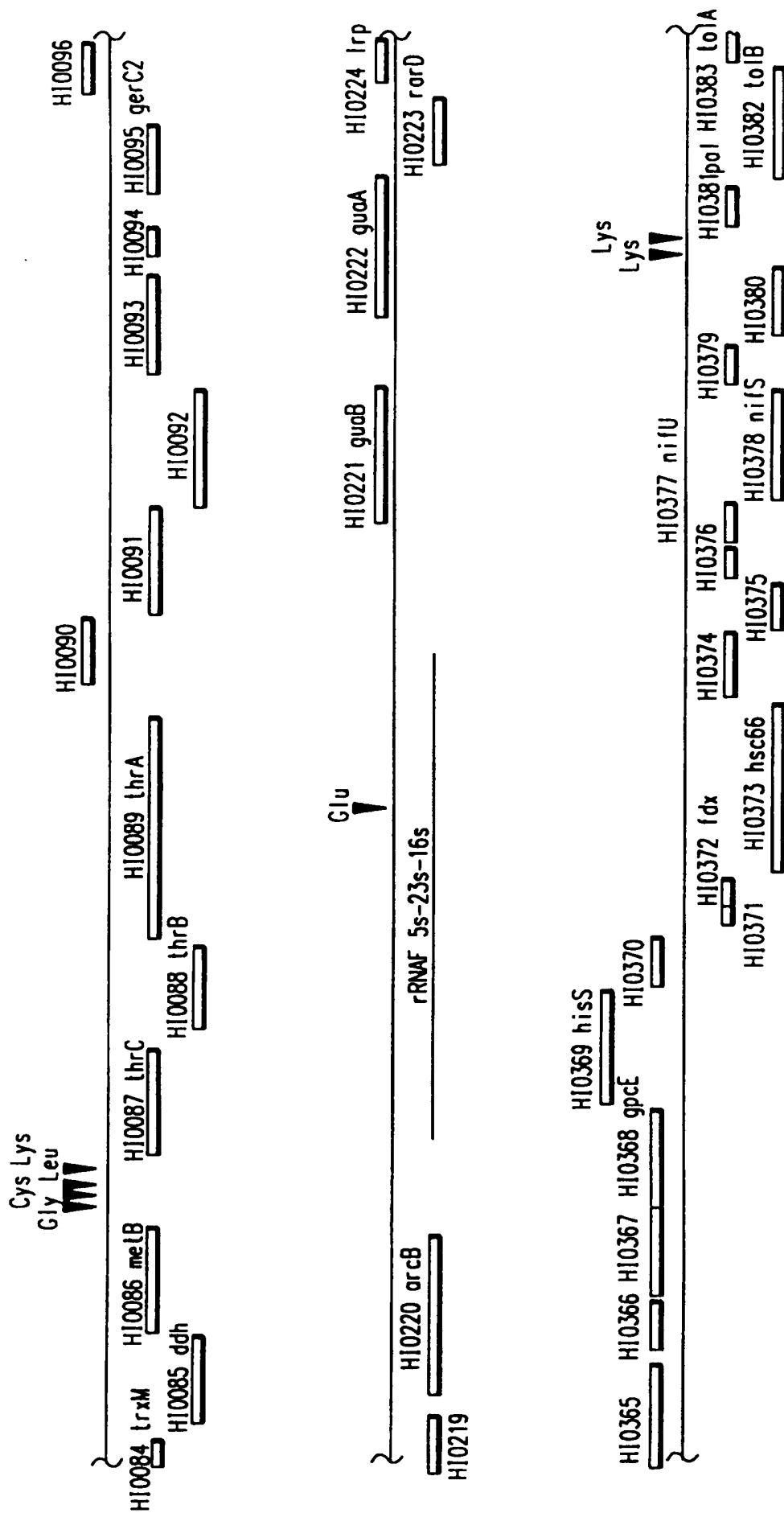


FIG.6G

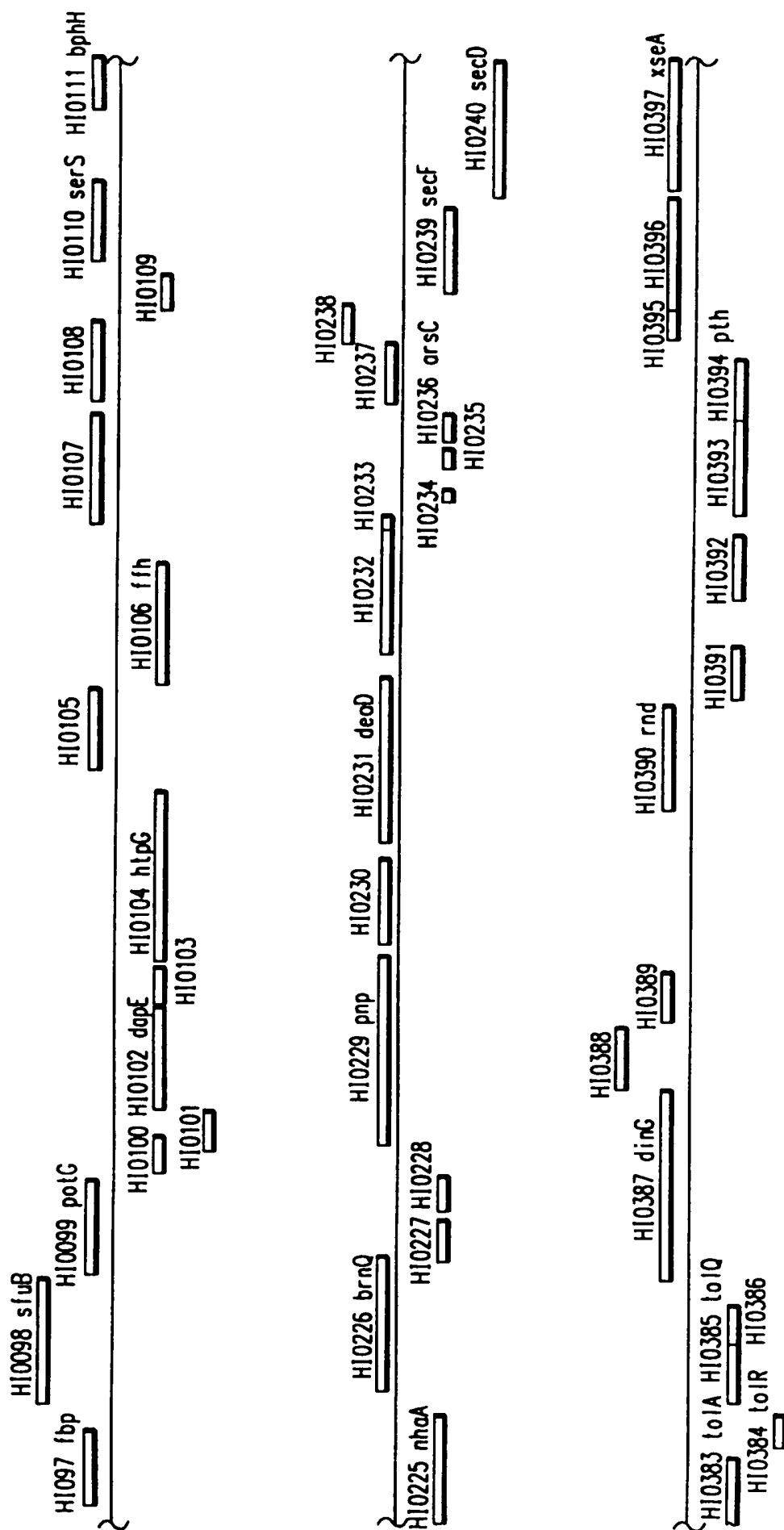


FIG. 6H

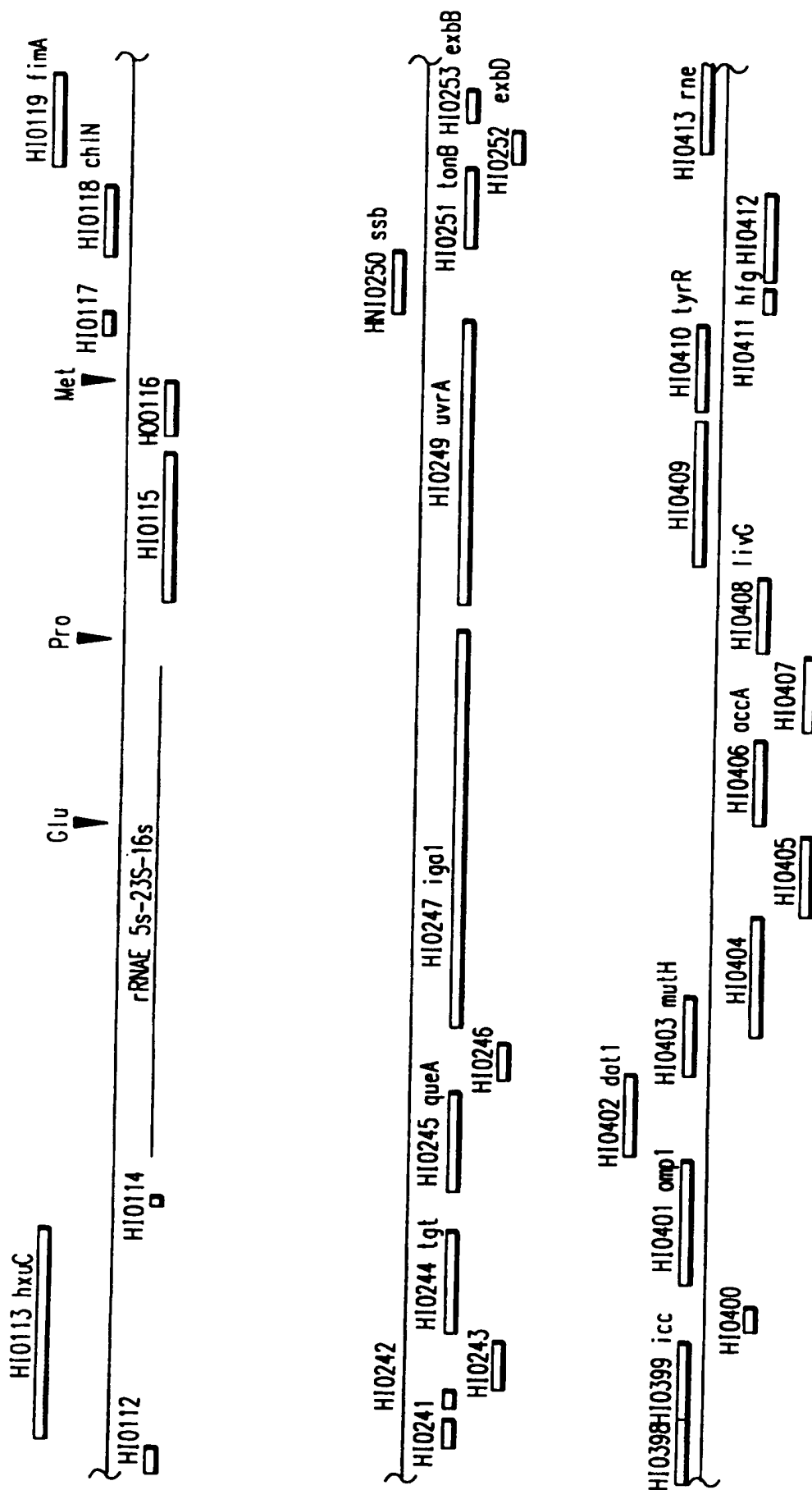


FIG. 6I

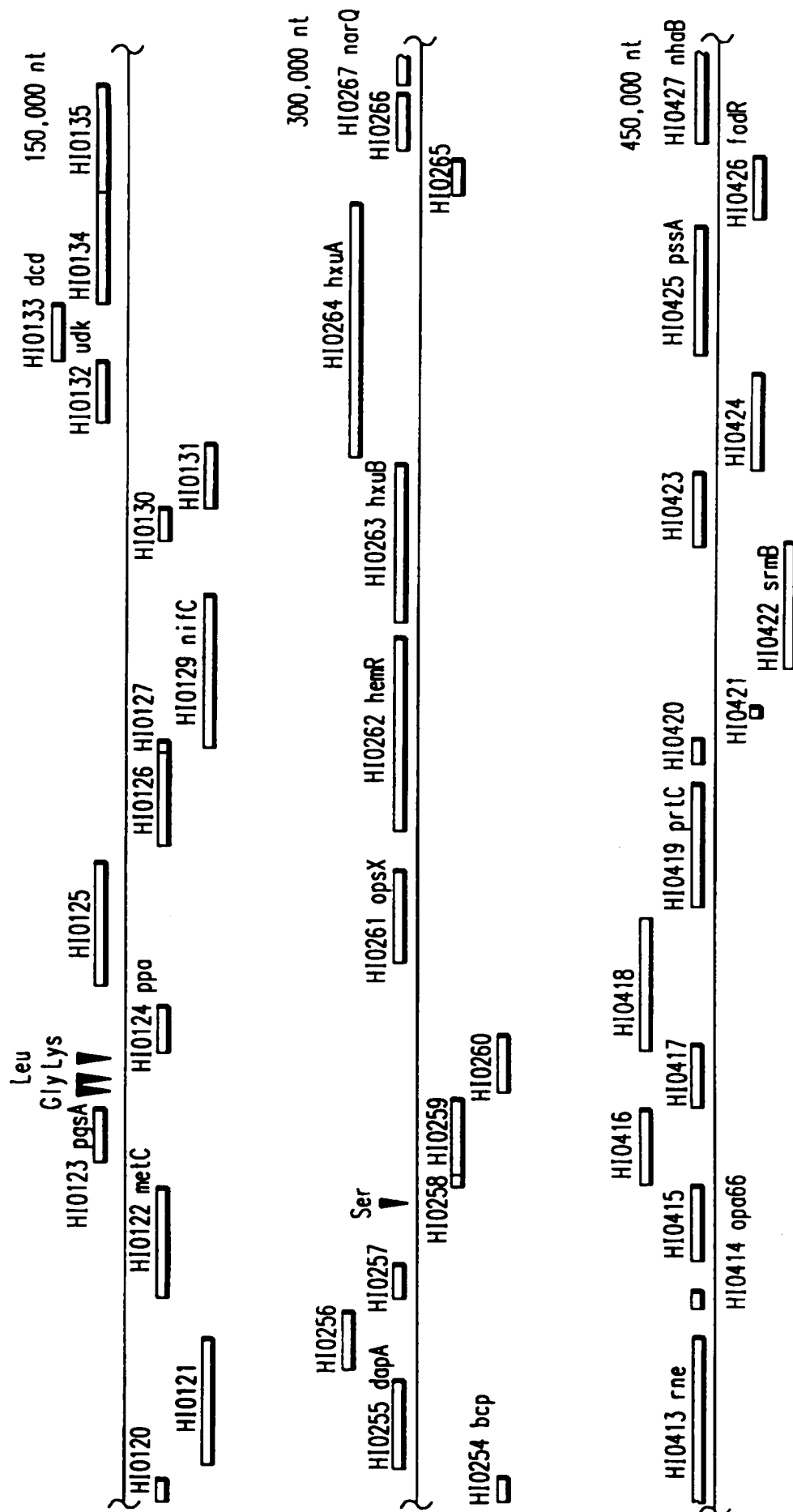


FIG.6J

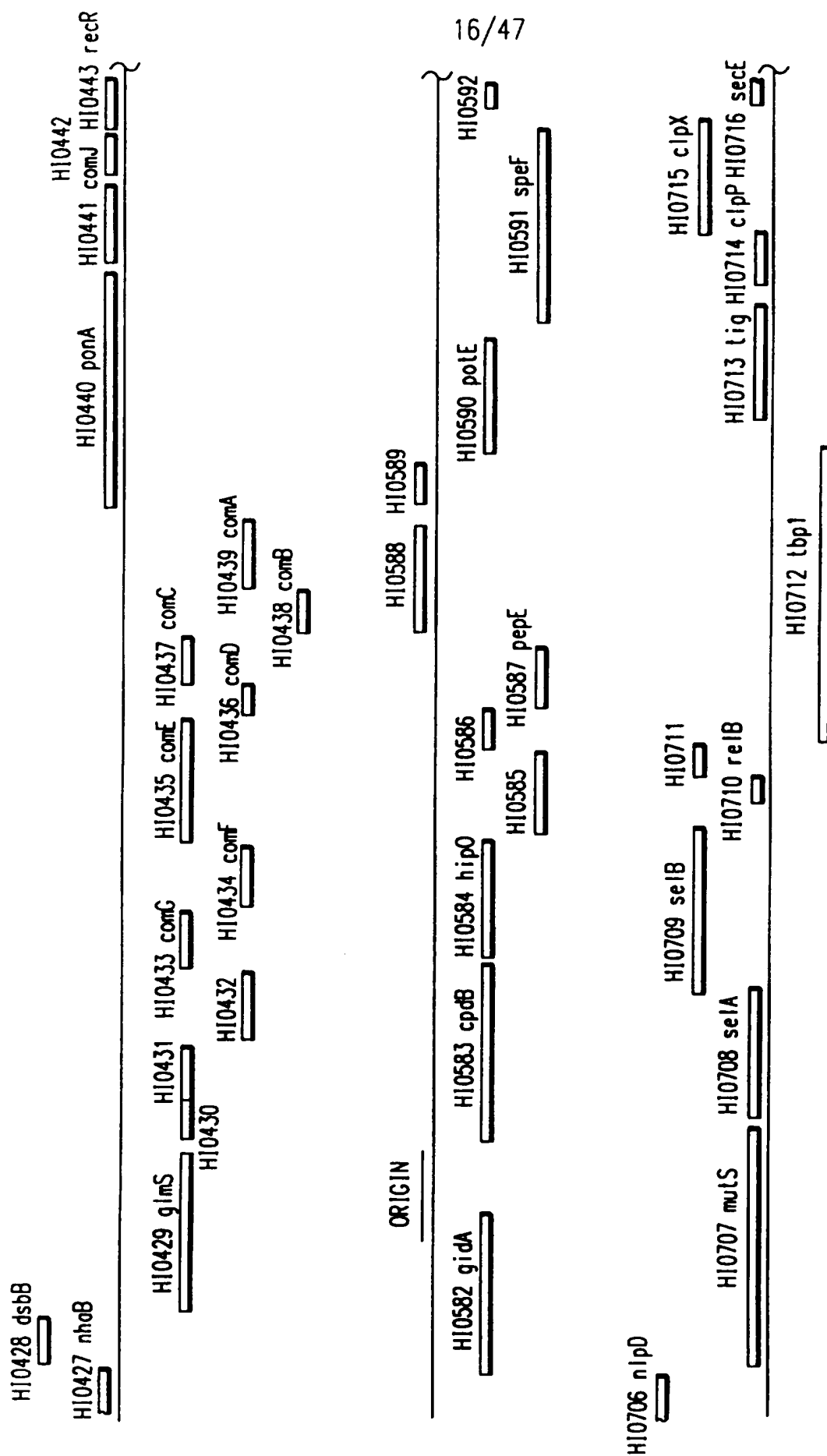


FIG. 6K

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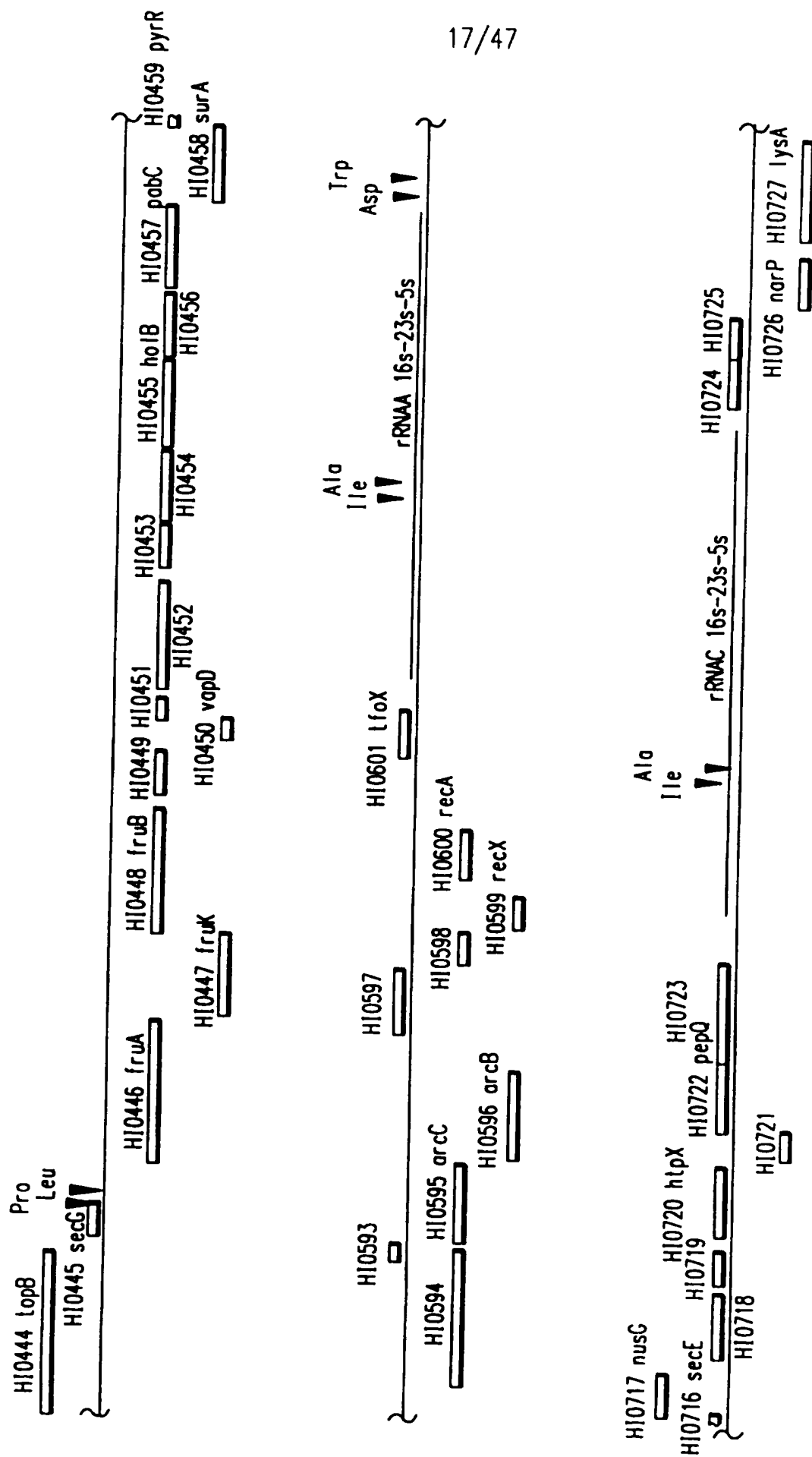


FIG. 6L

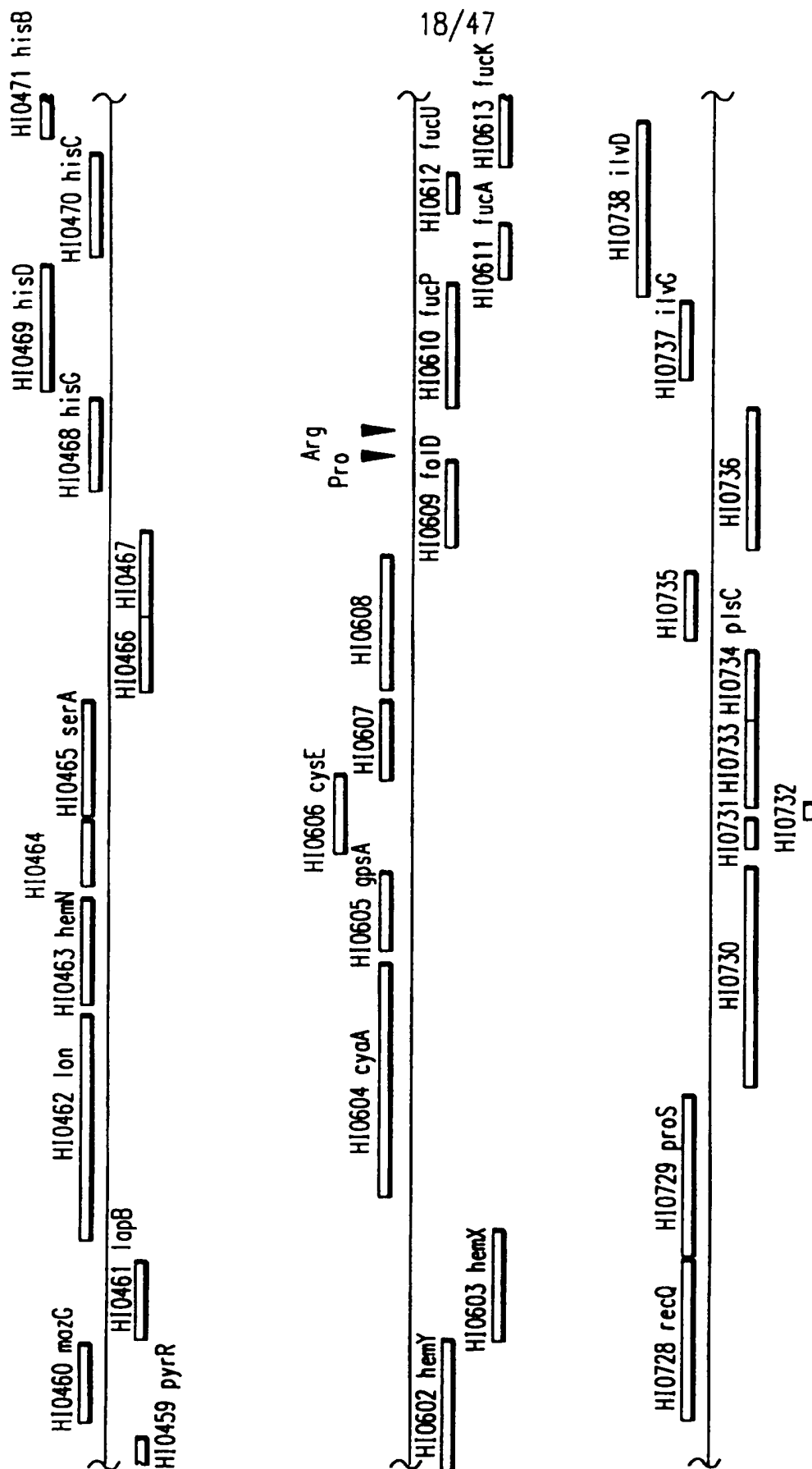


FIG.6M

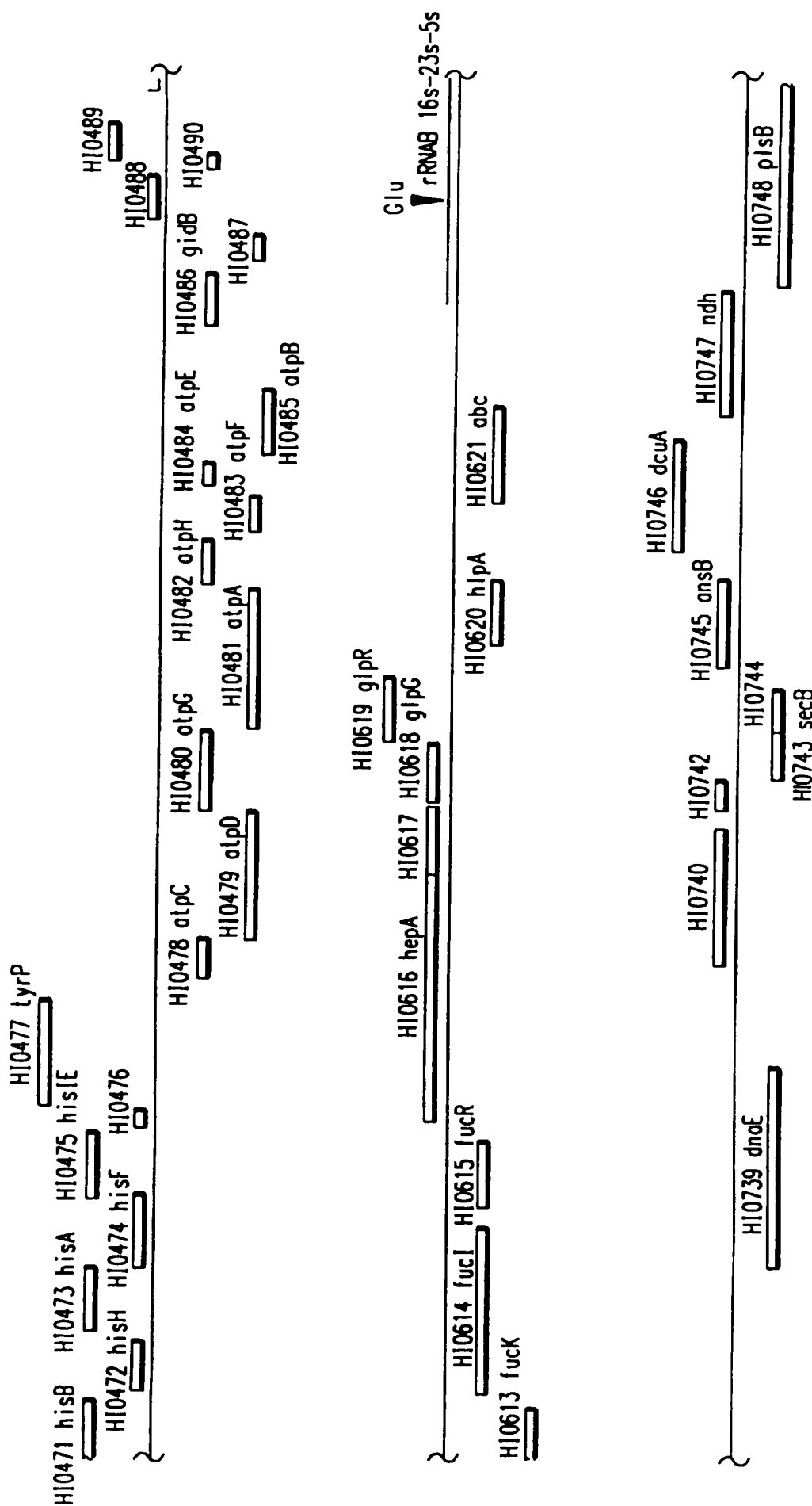


FIG. 6N

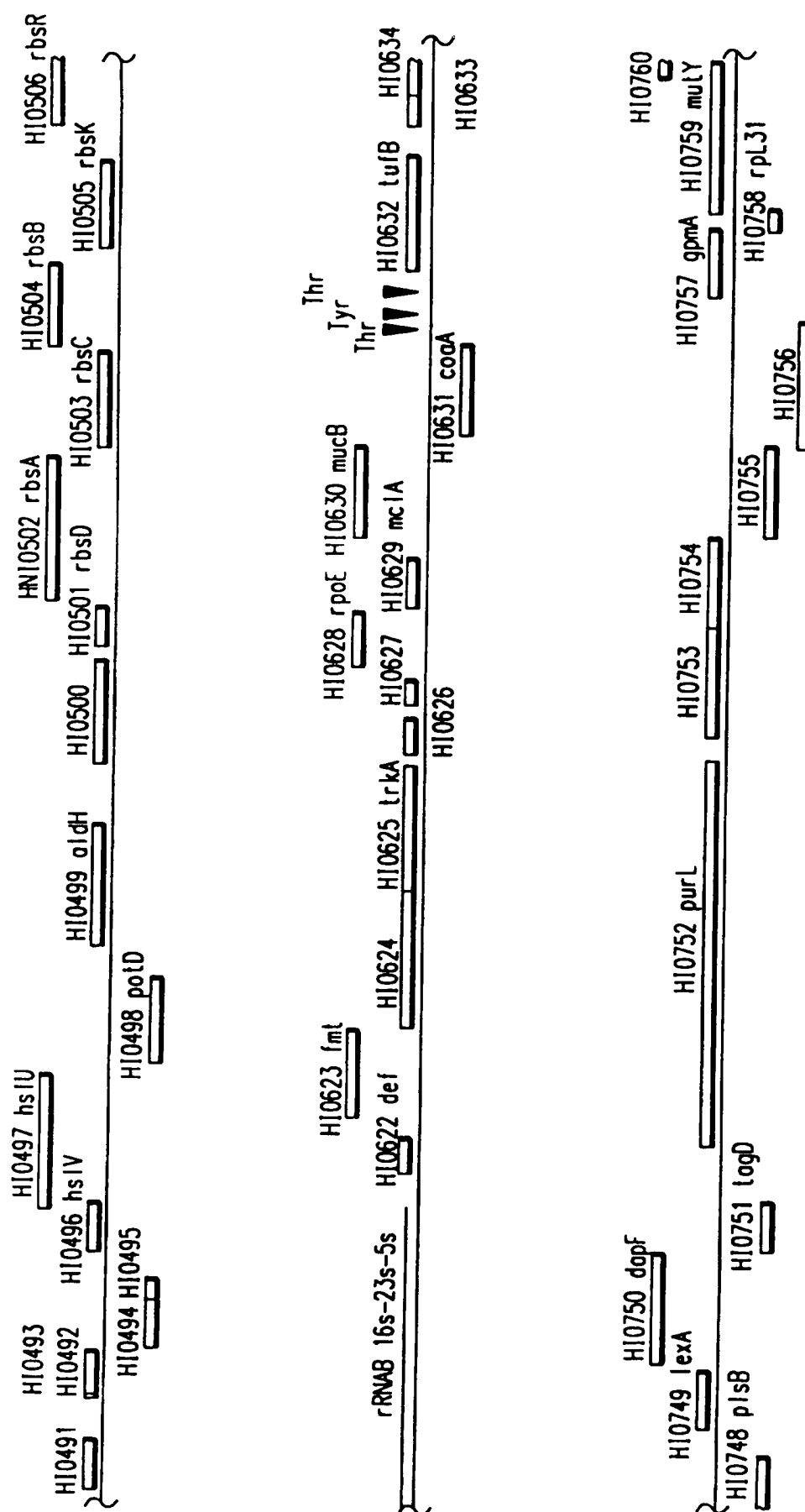


FIG.60

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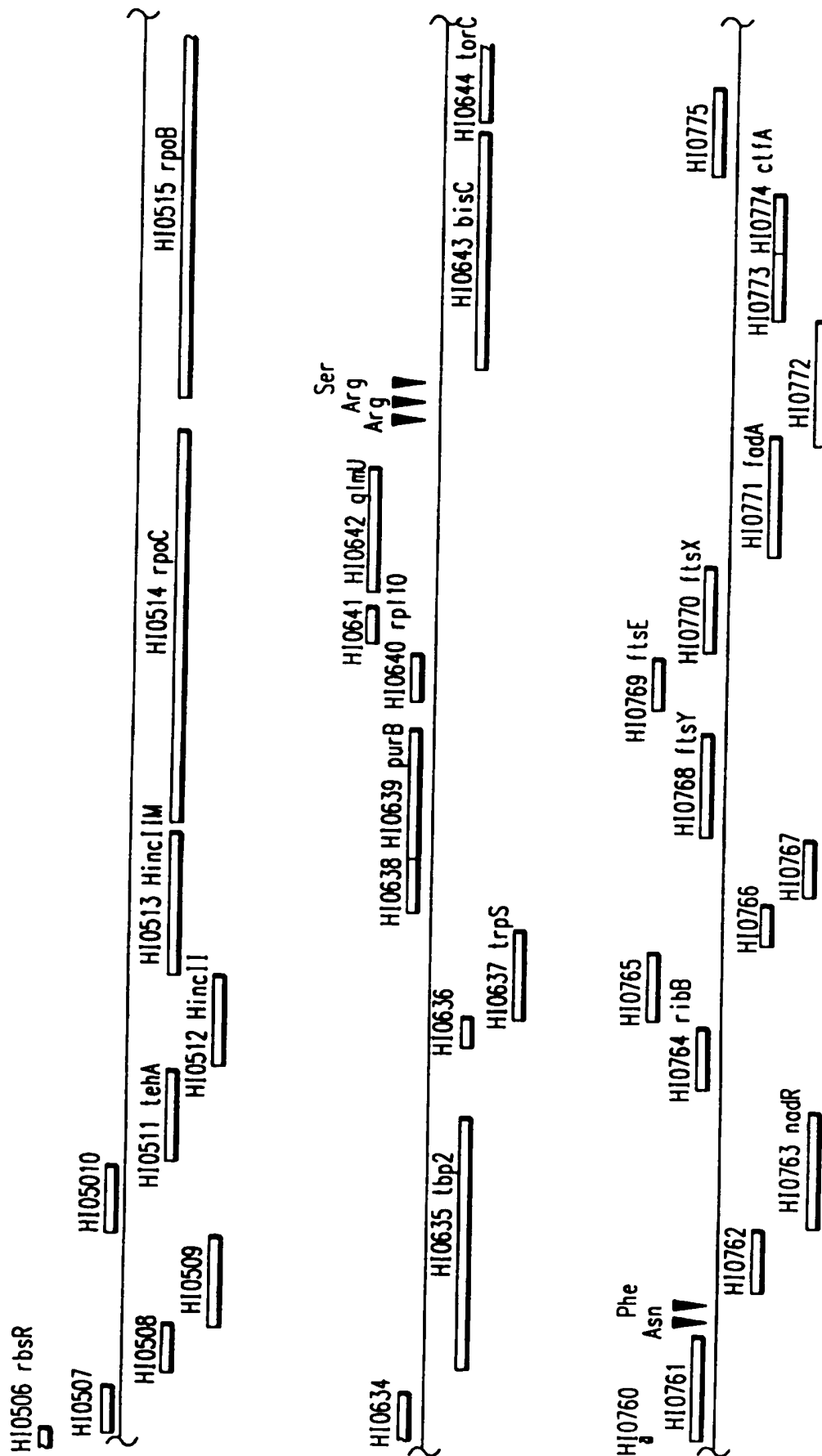


FIG. 6P

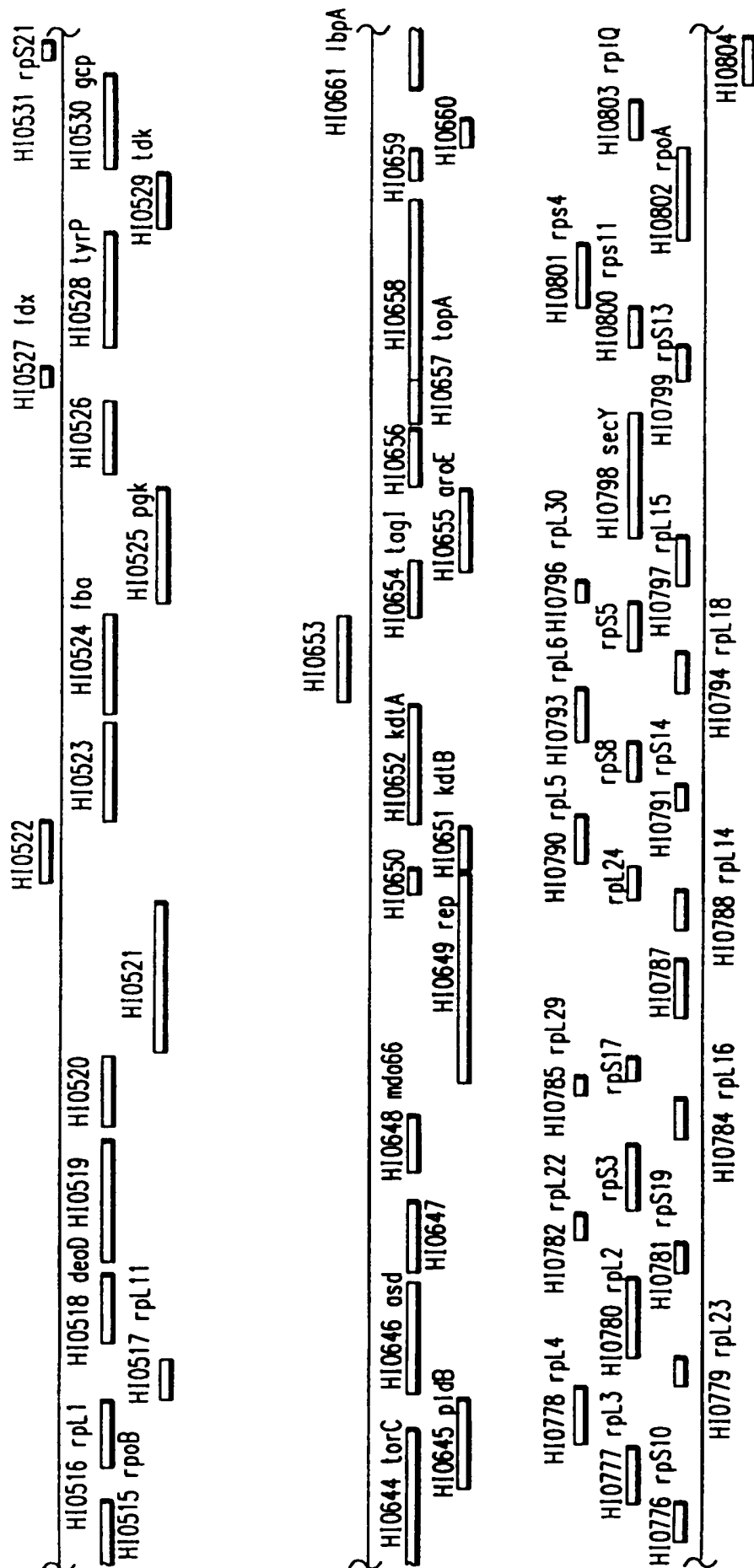


FIG.6Q

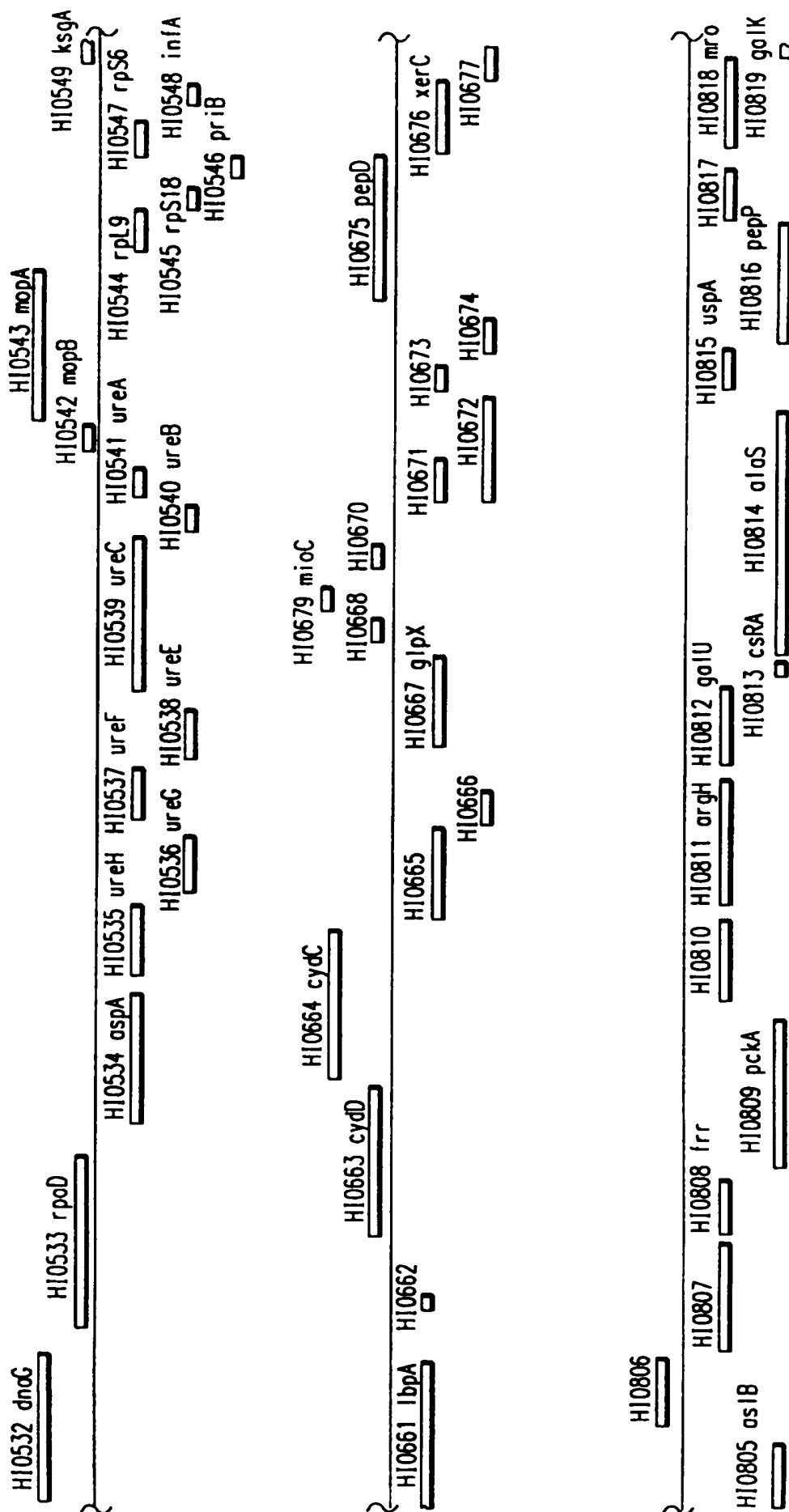


FIG.6R

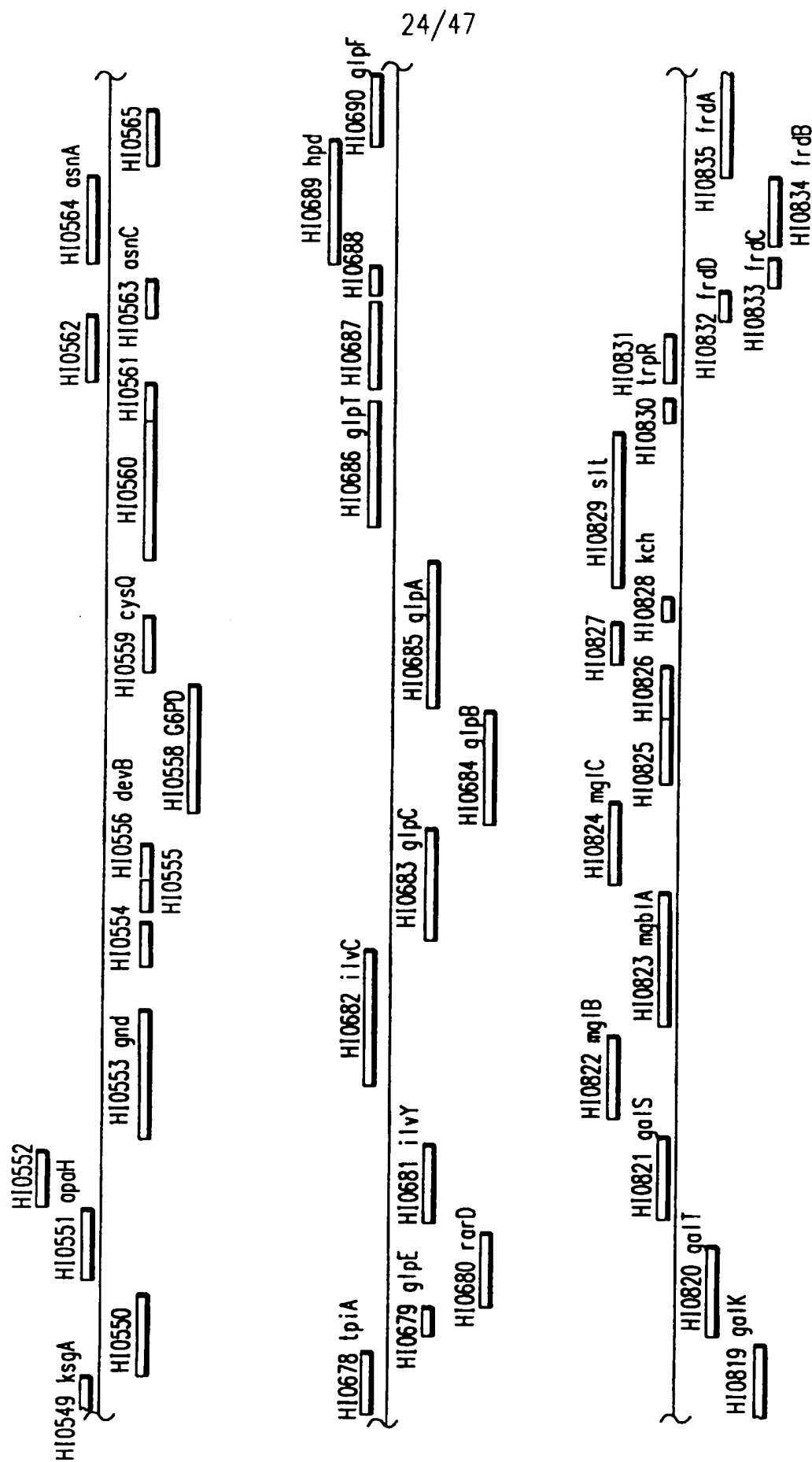


FIG. 6S

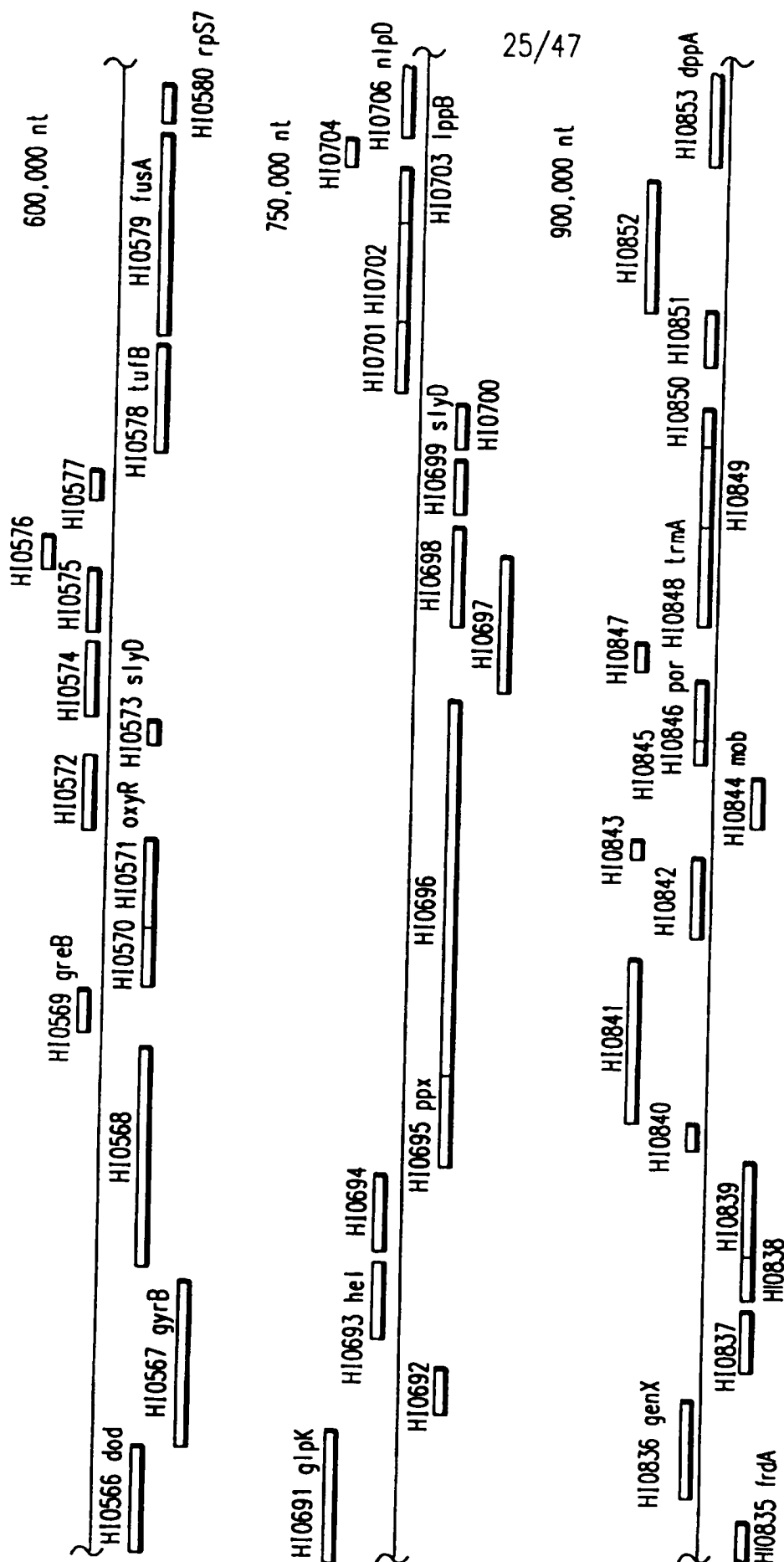


FIG.6T

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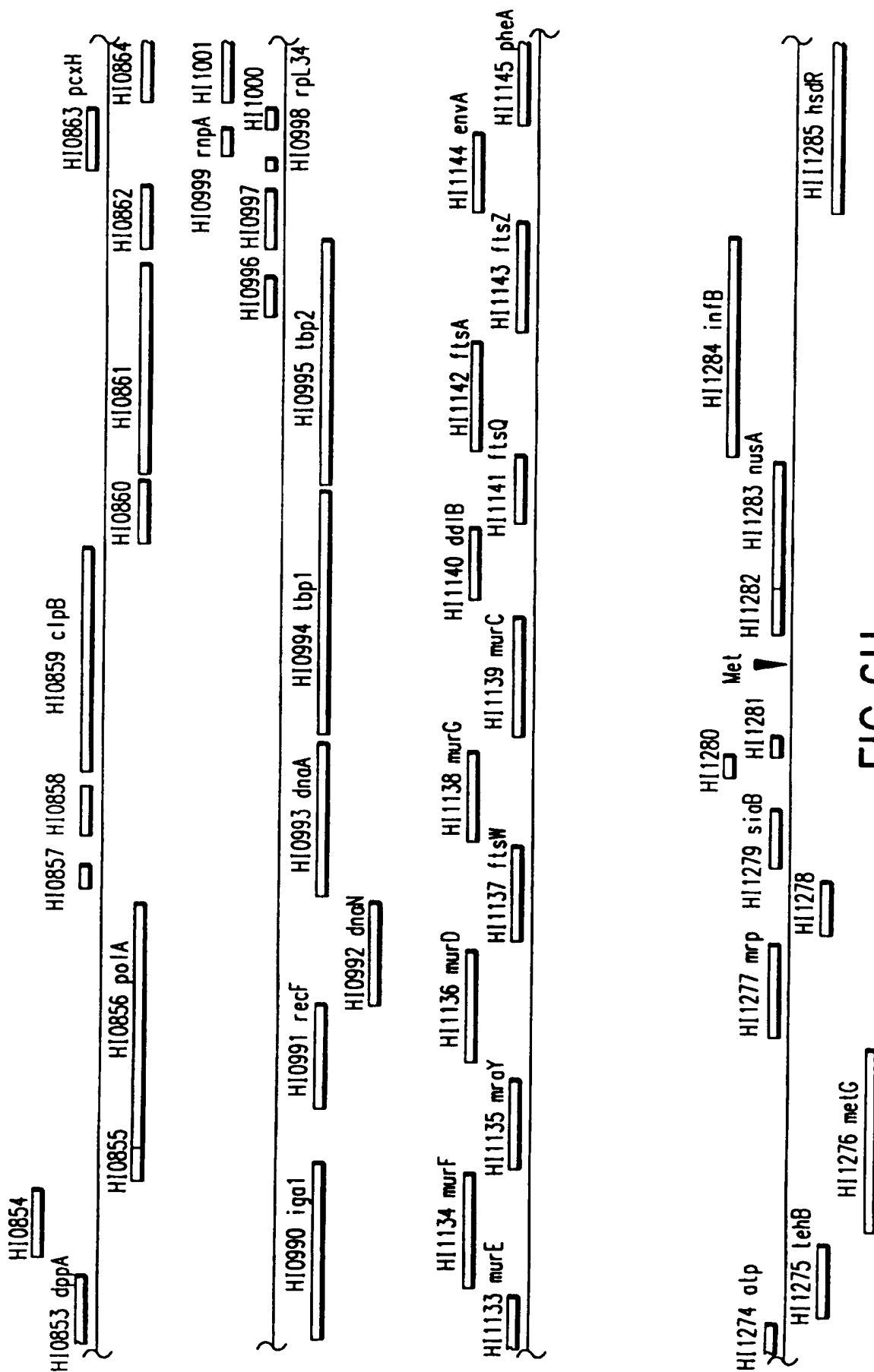


FIG. 6U

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FIG. 6V

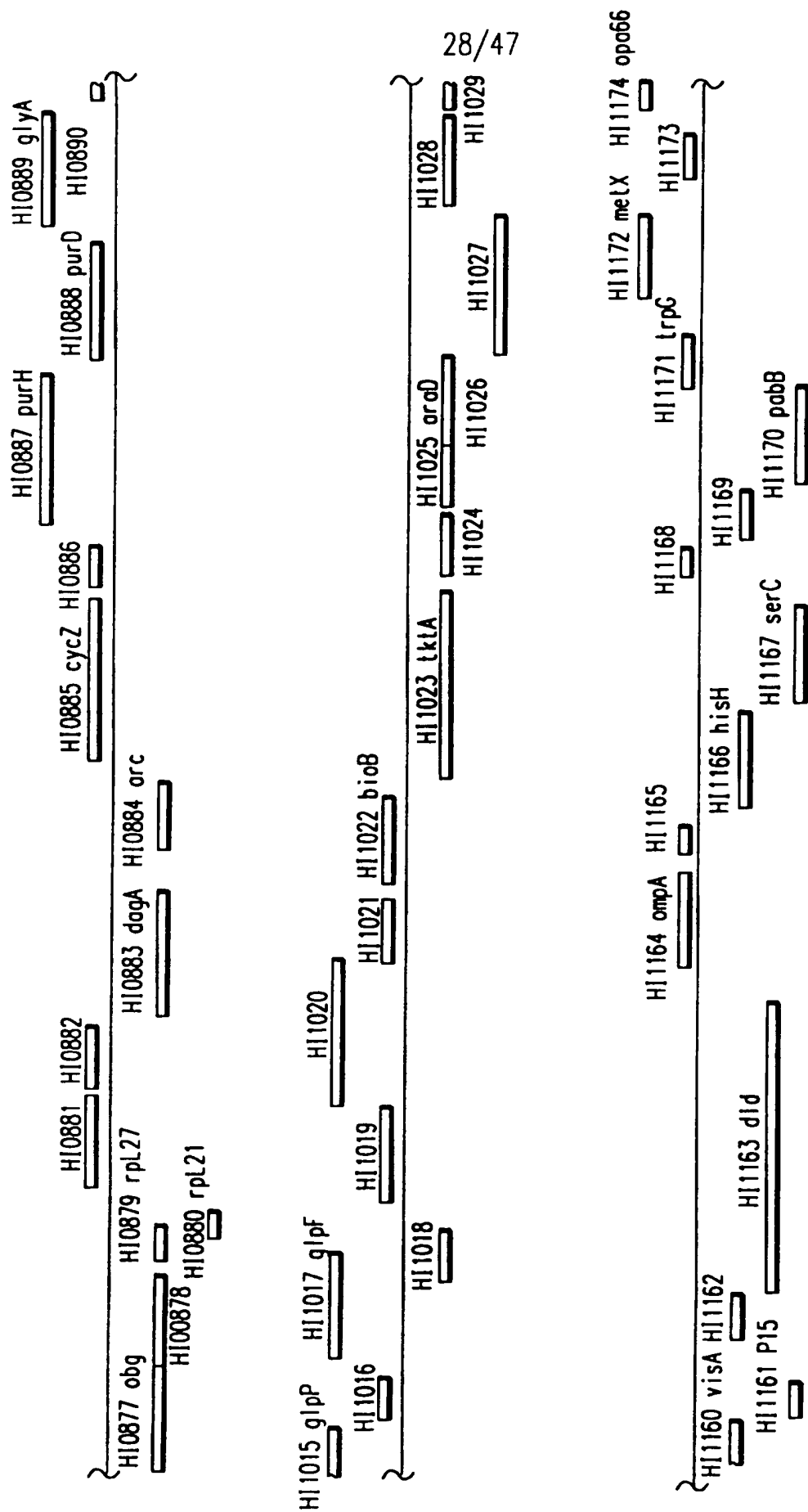


FIG. 6W

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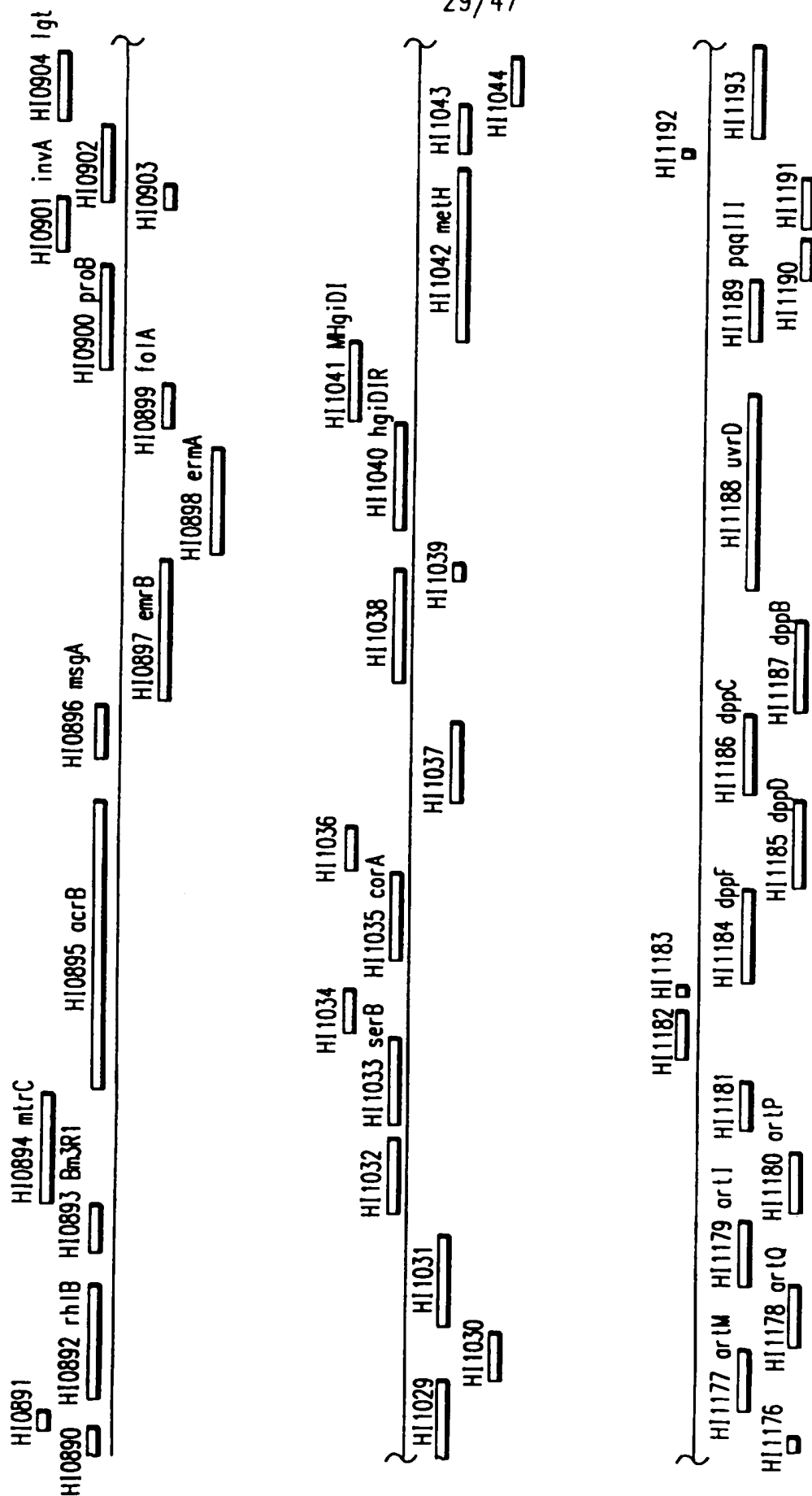


FIG.6X

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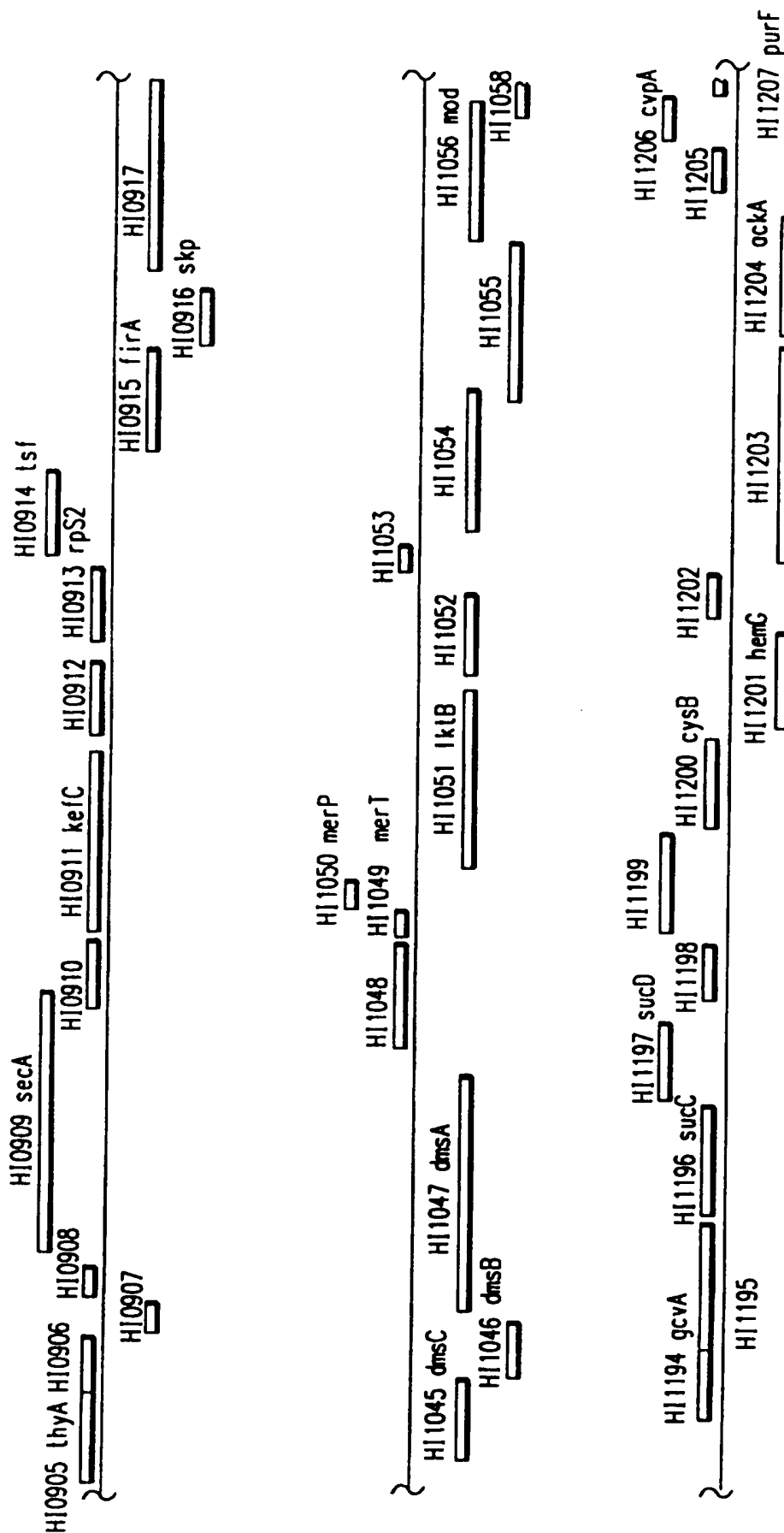


FIG.6Y

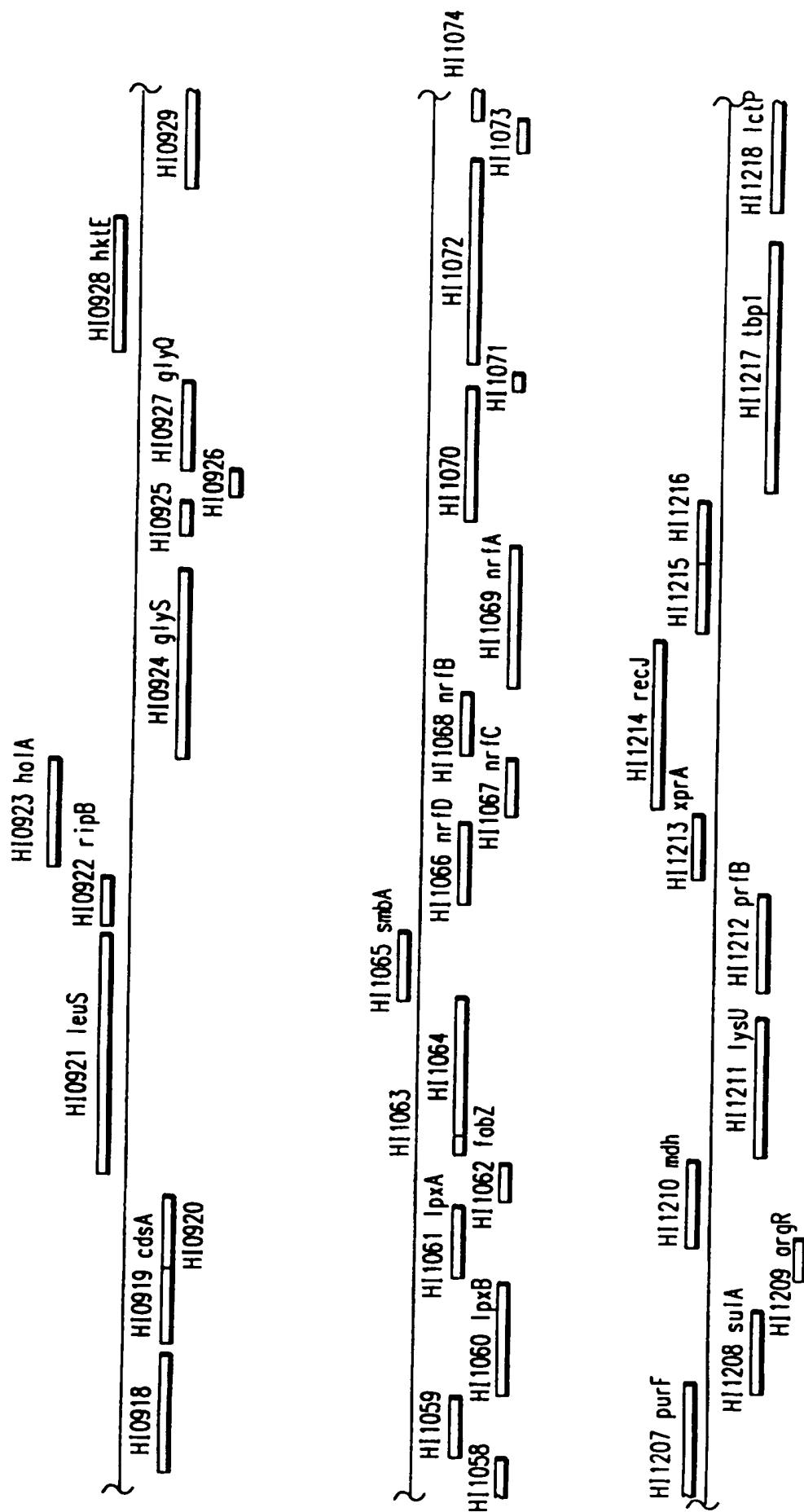


FIG.6Z

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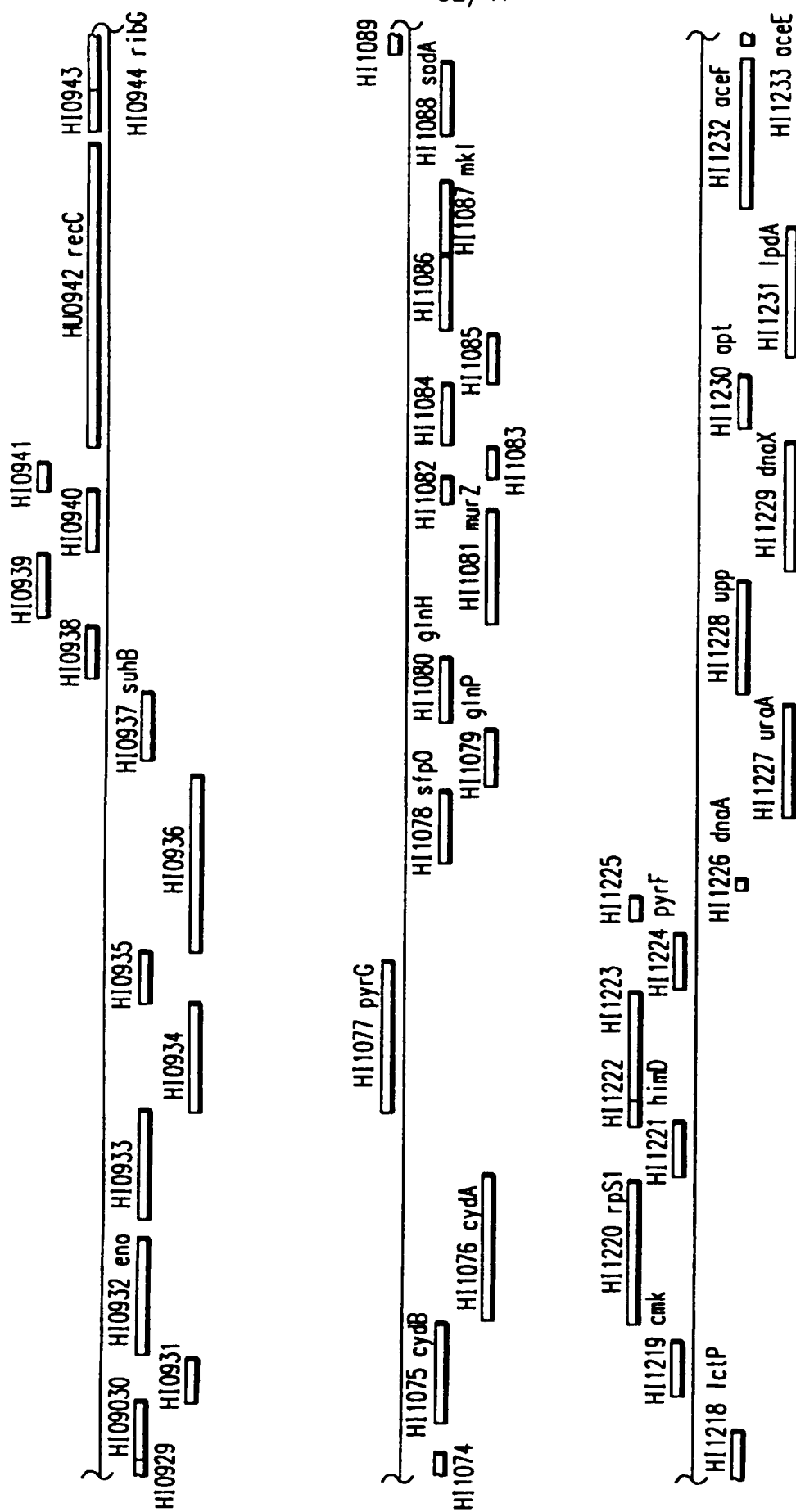


FIG. 6AA

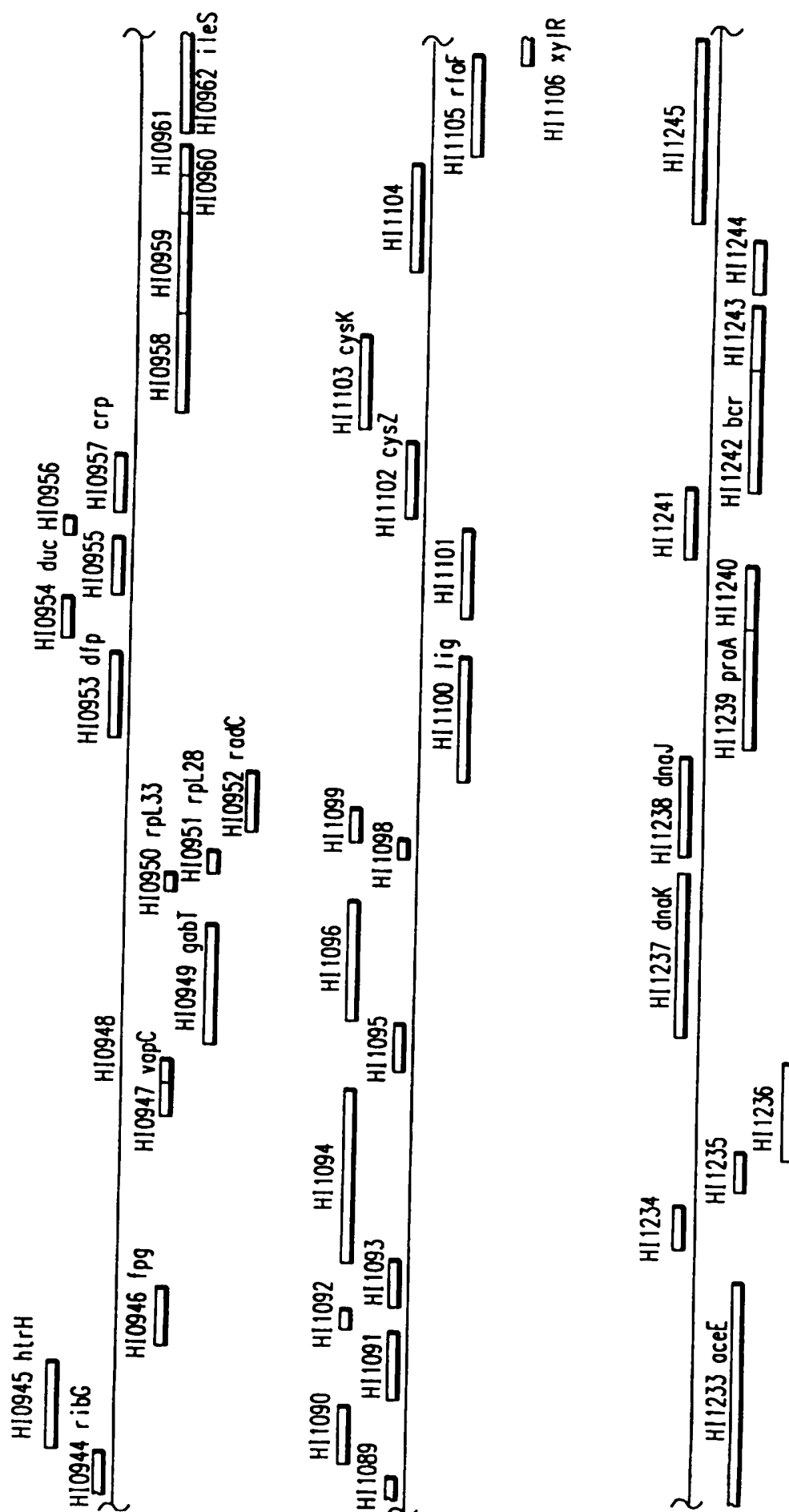


FIG. 6AB

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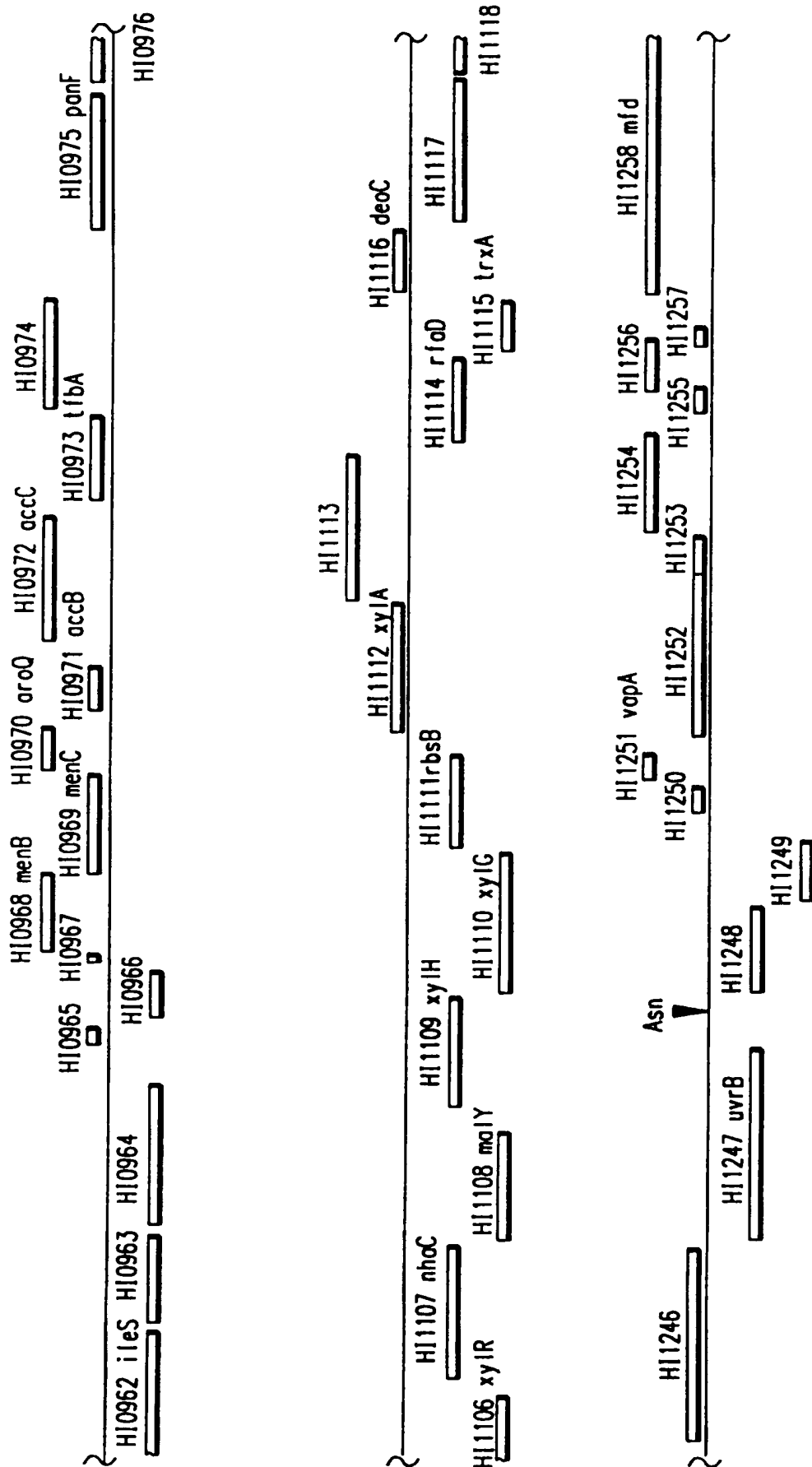


FIG. 6AC

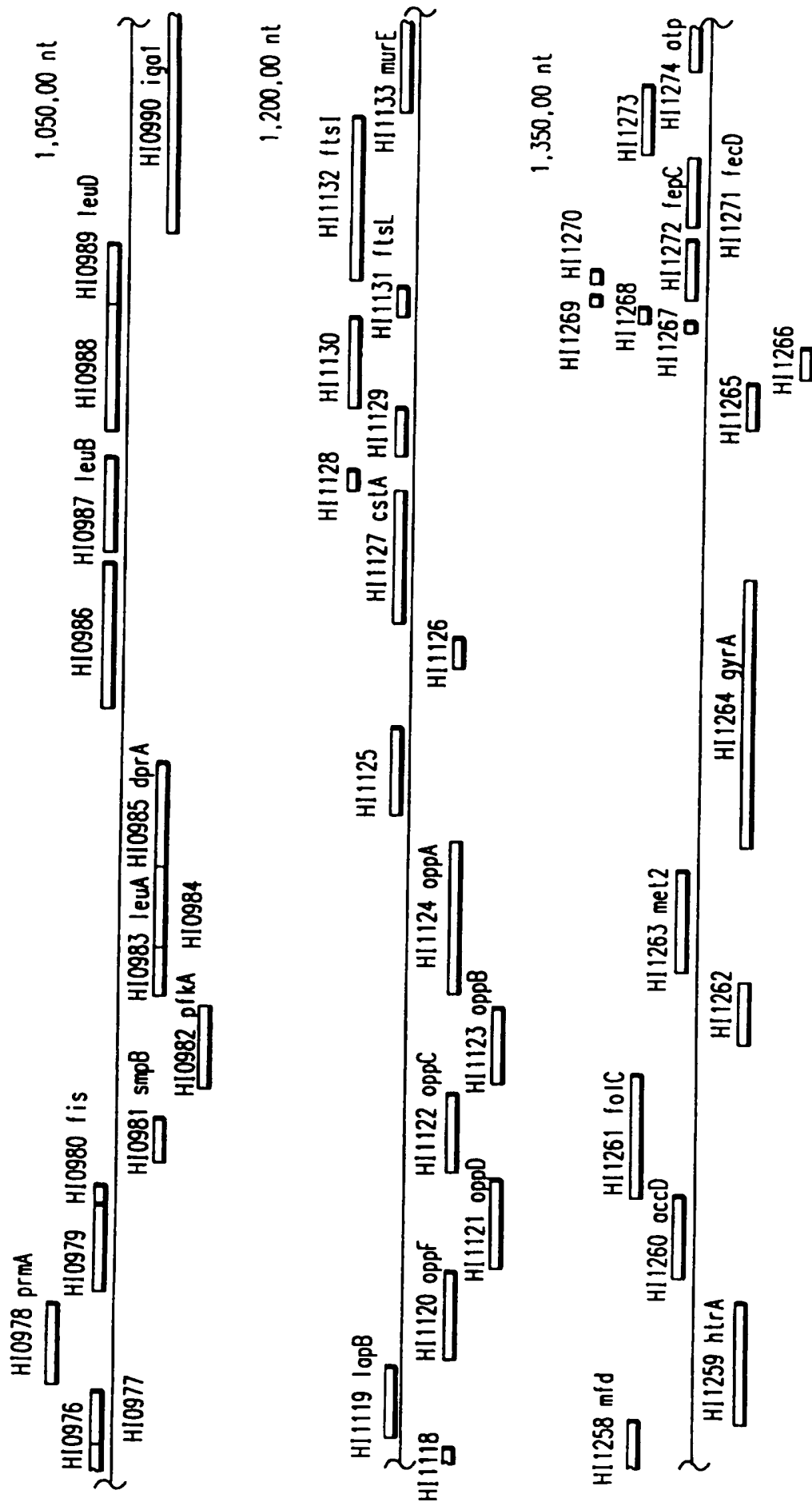


FIG. 6AD

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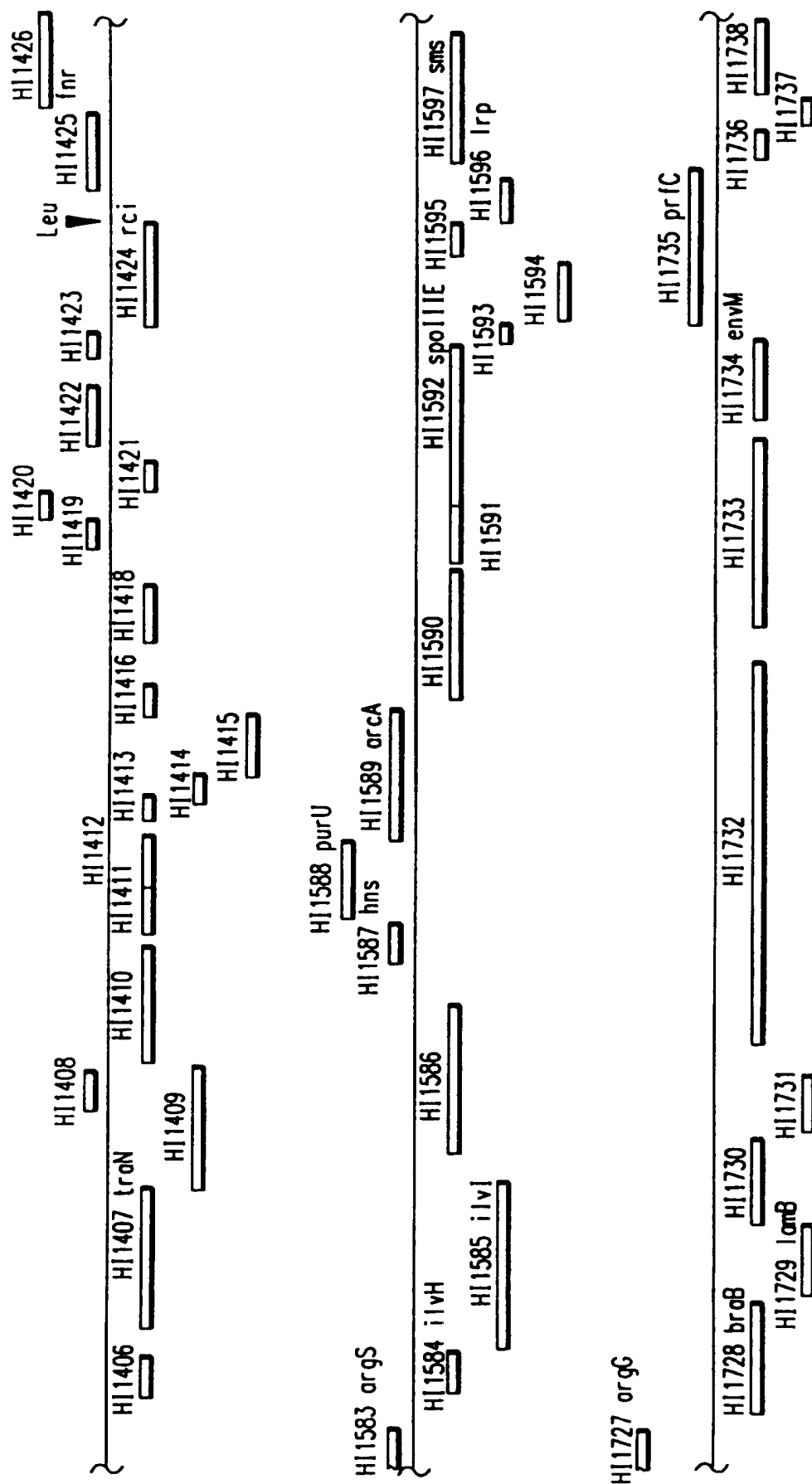


FIG. 6AE

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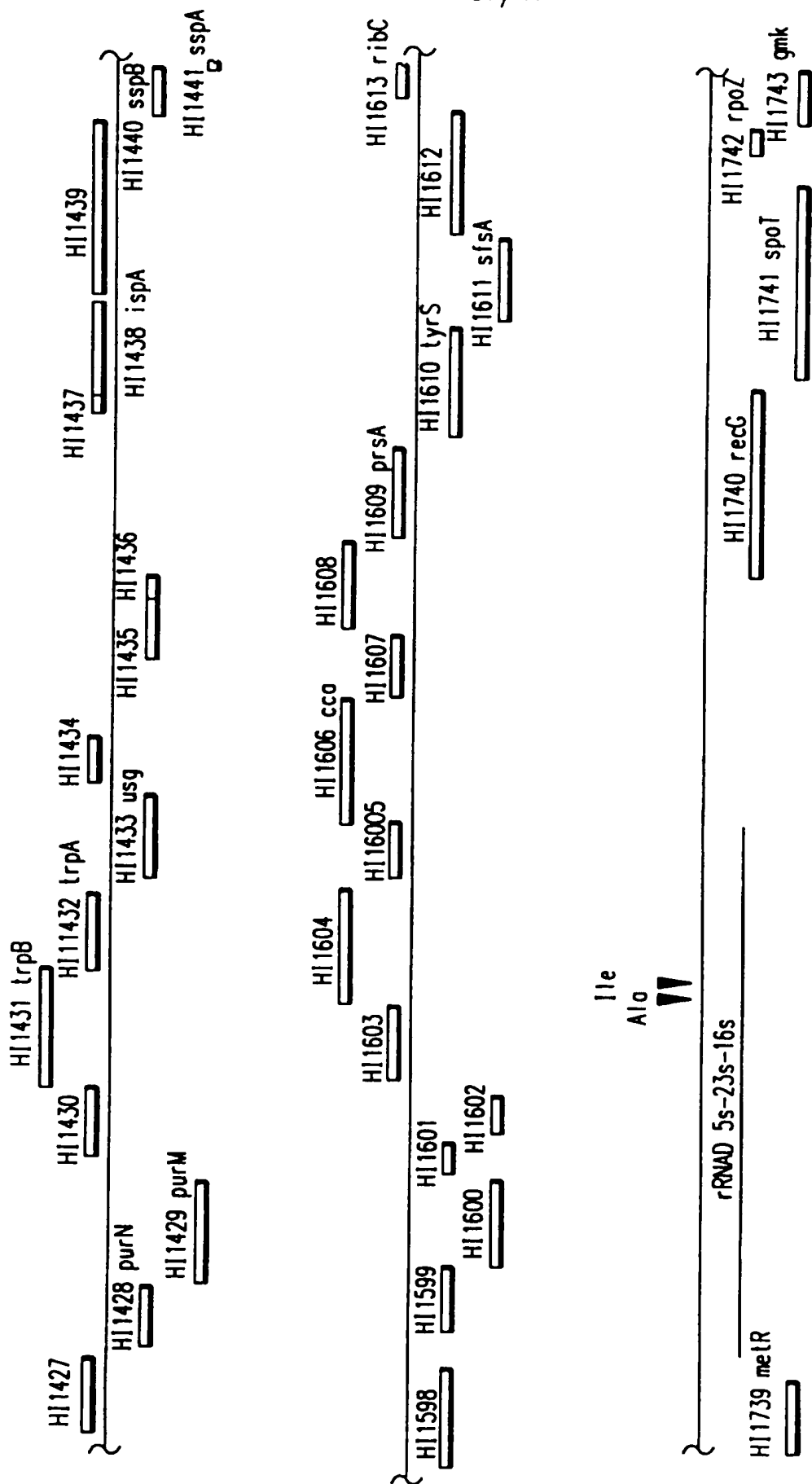


FIG. 6AF

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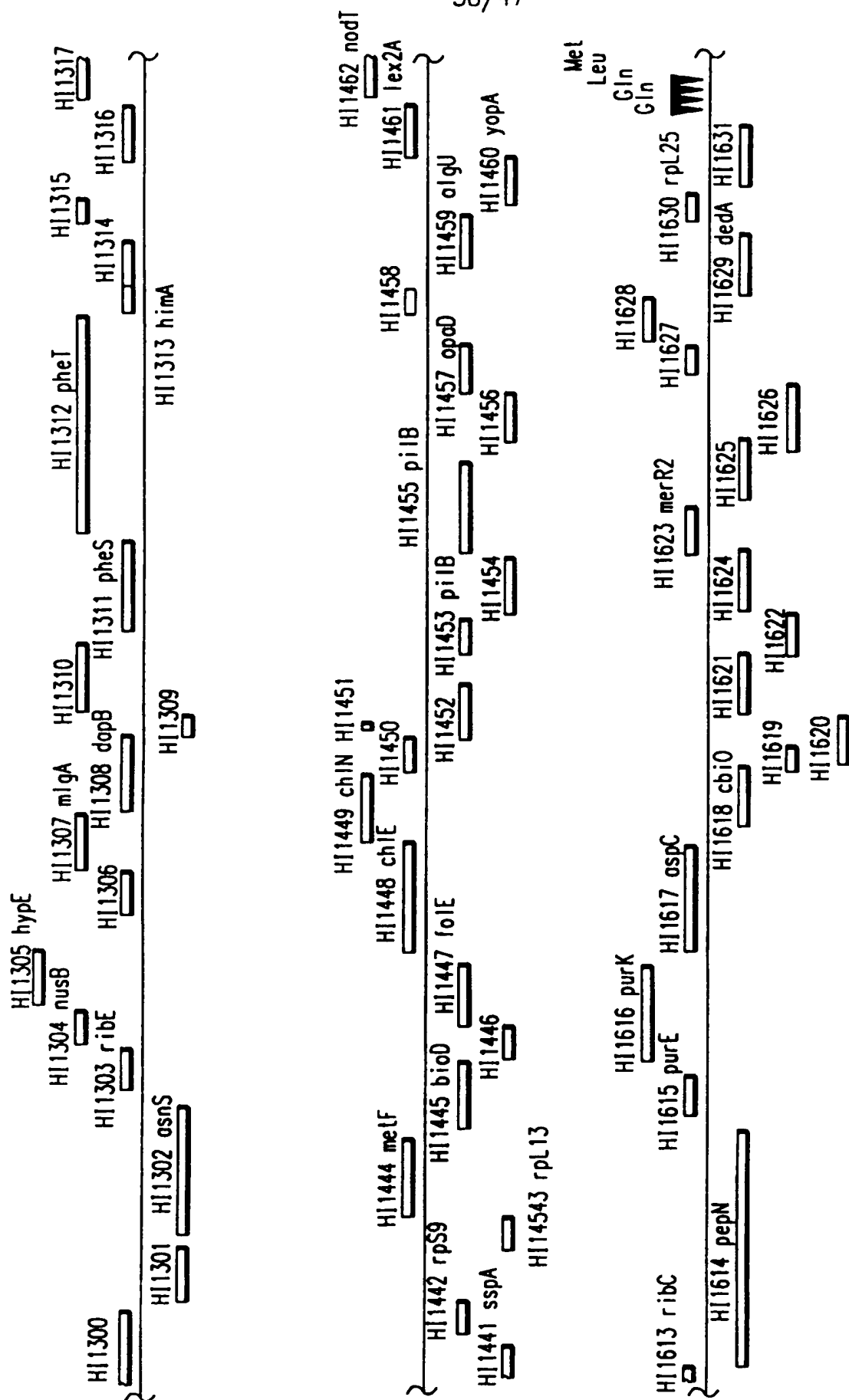


FIG. 6AG

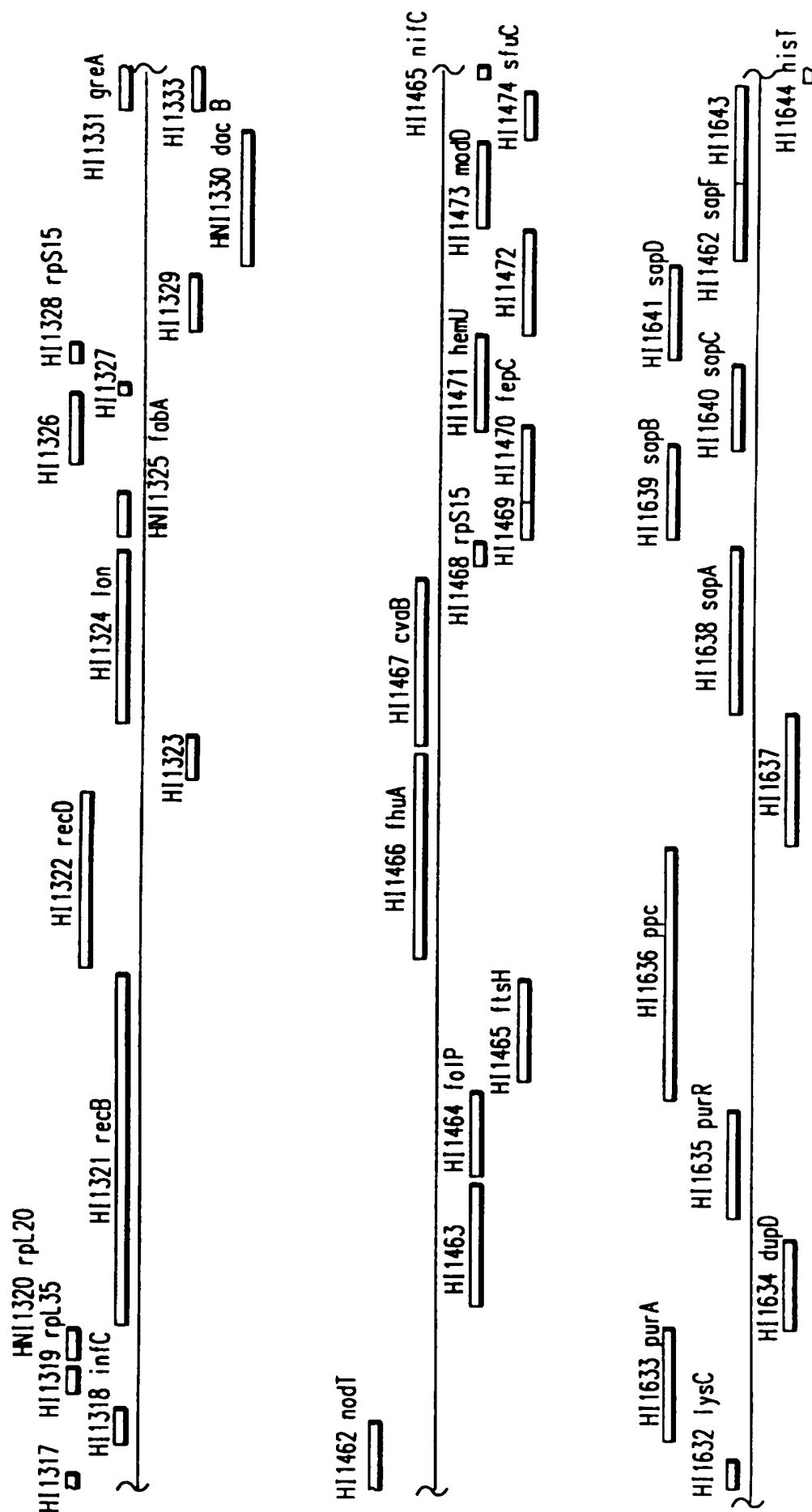


FIG. 6AH

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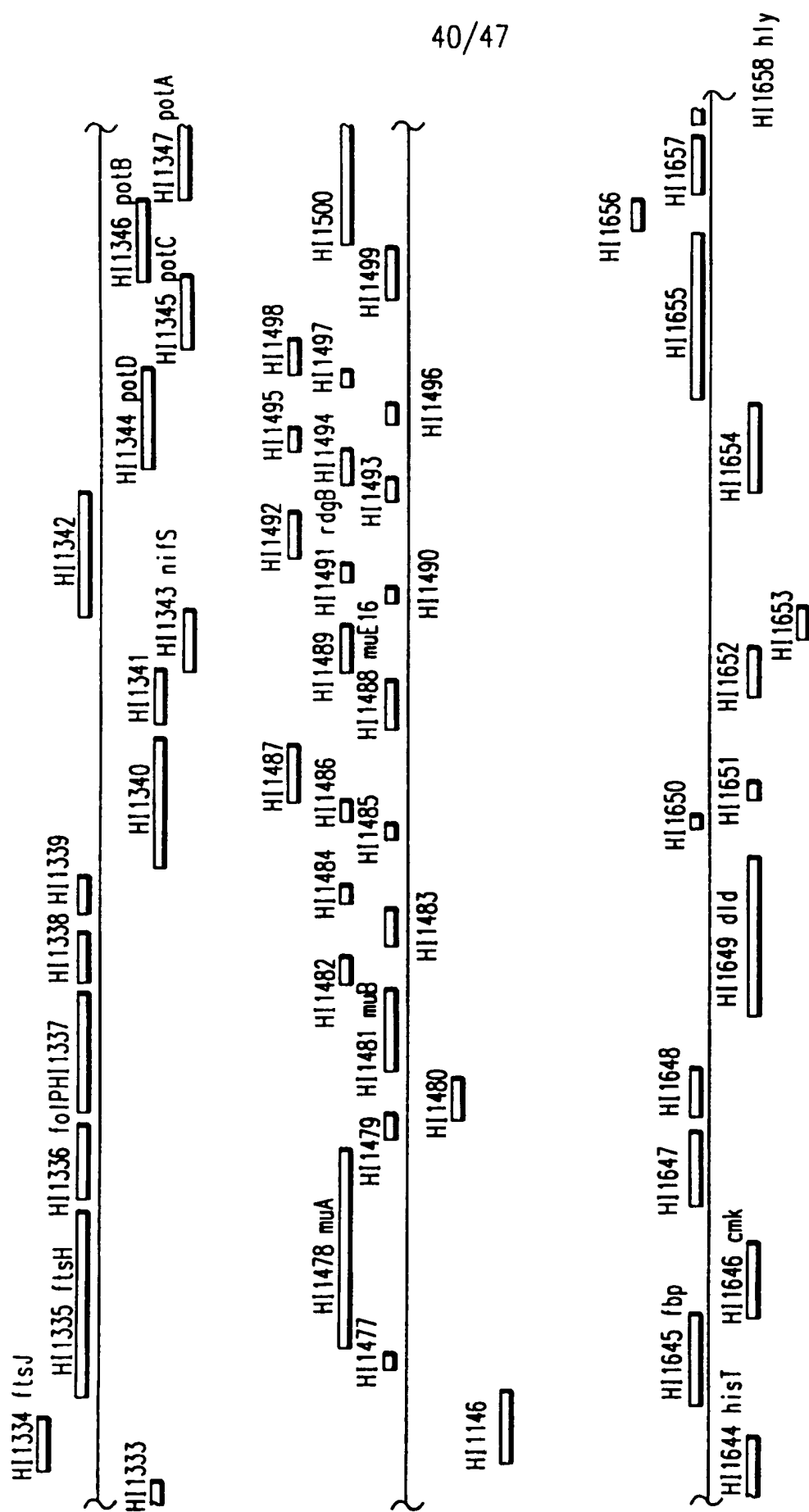


FIG. 6AI

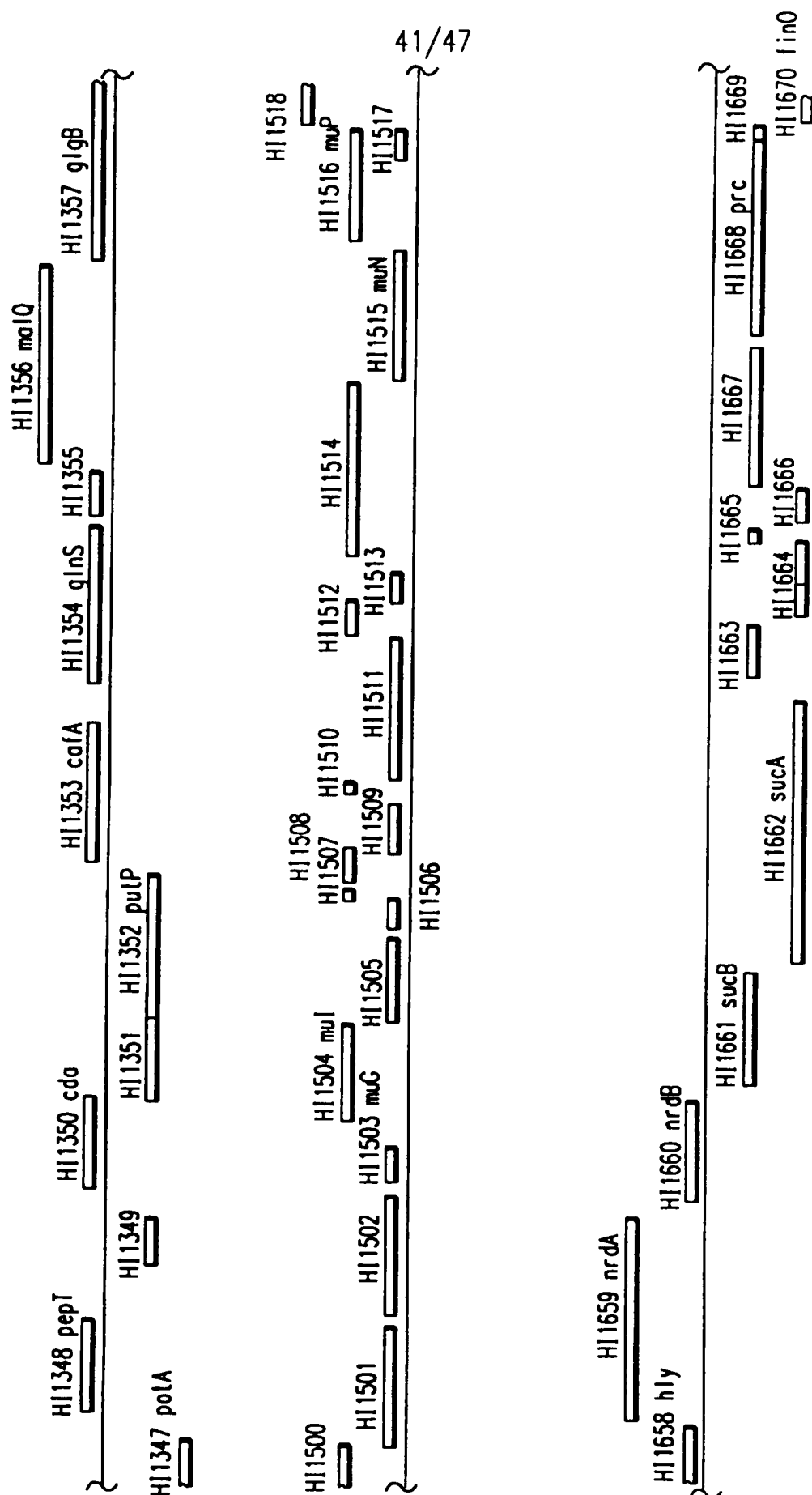


FIG. 6AJ

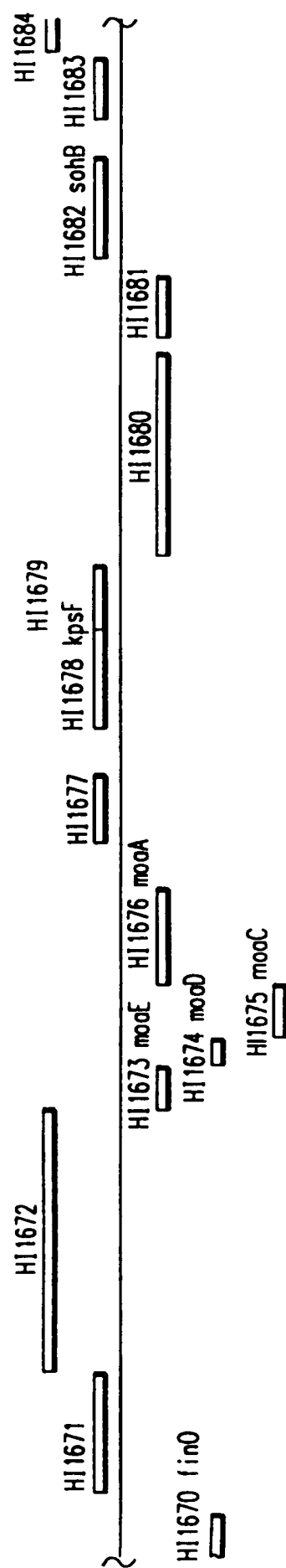
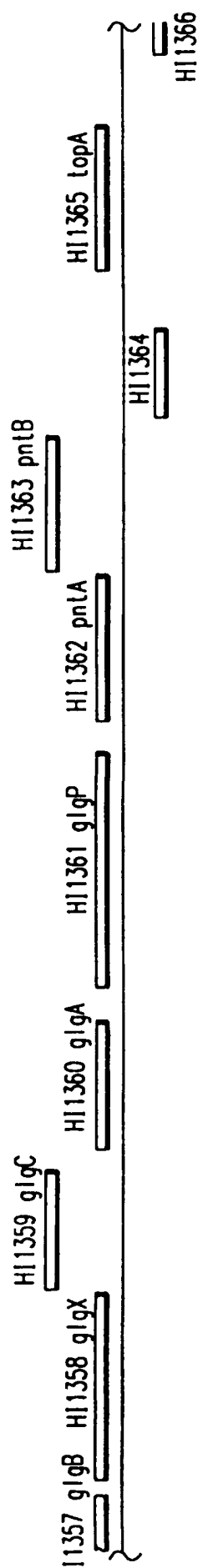


FIG. 6AK

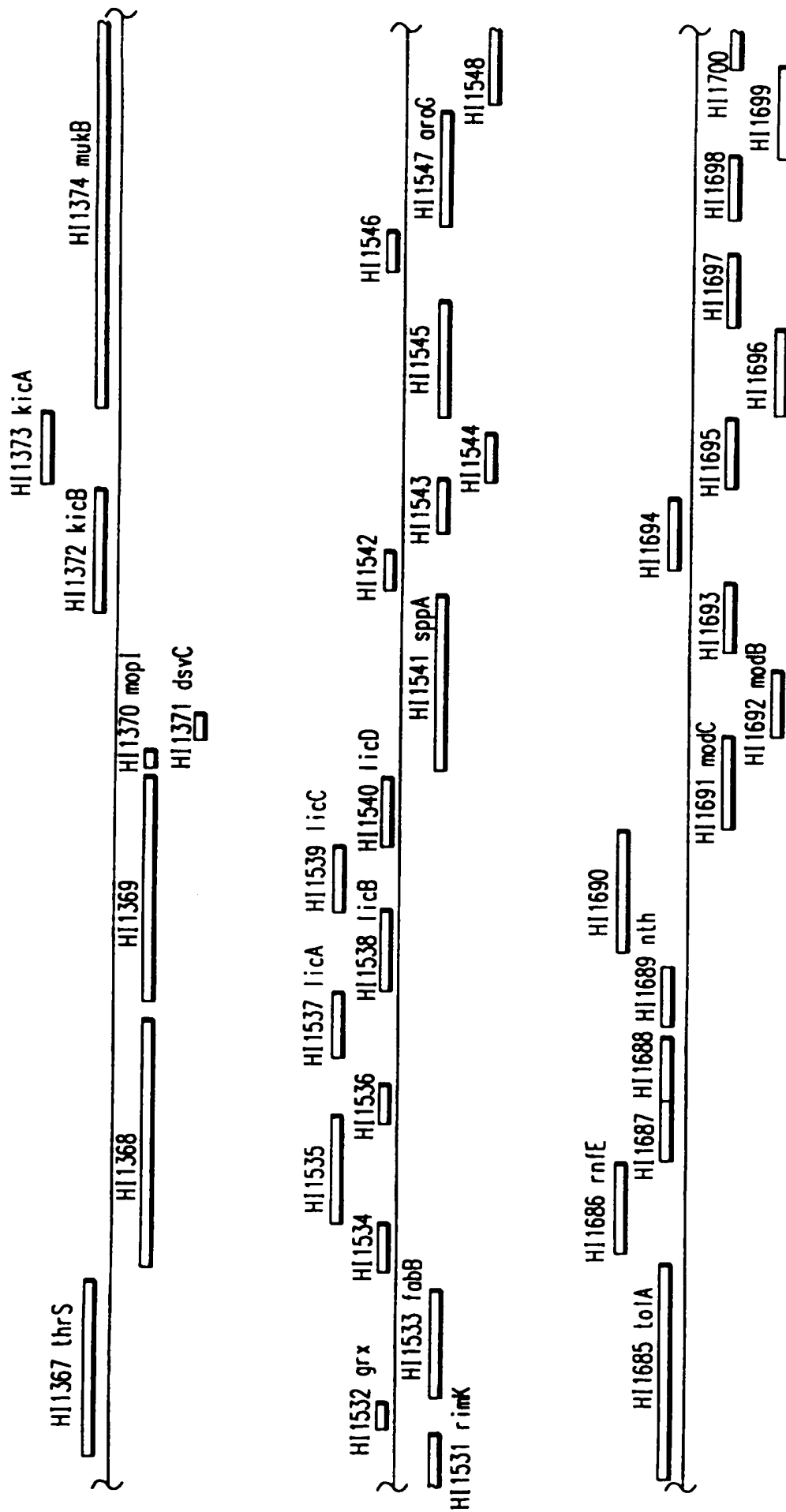


FIG.6AL

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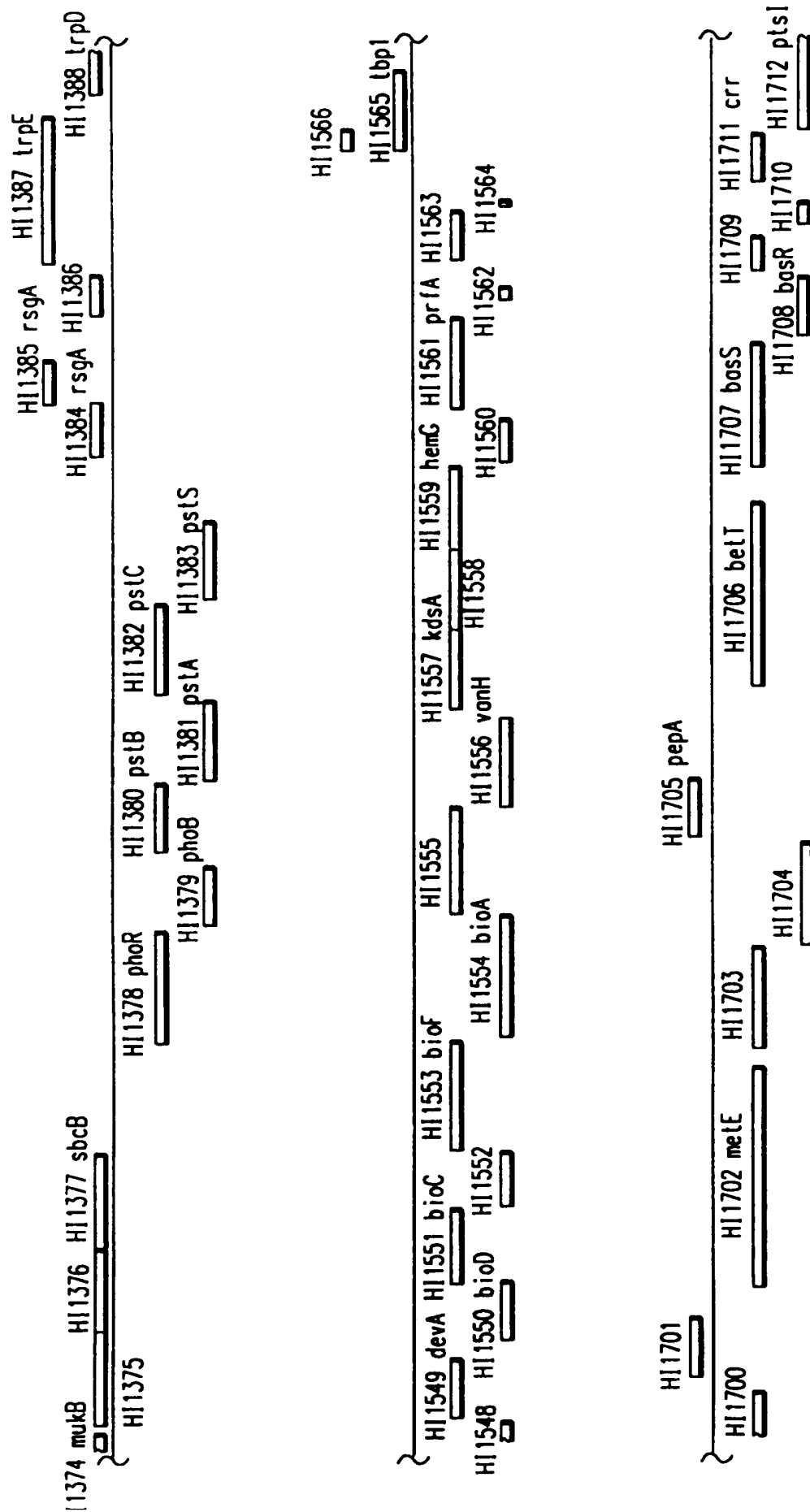


FIG. 6AM

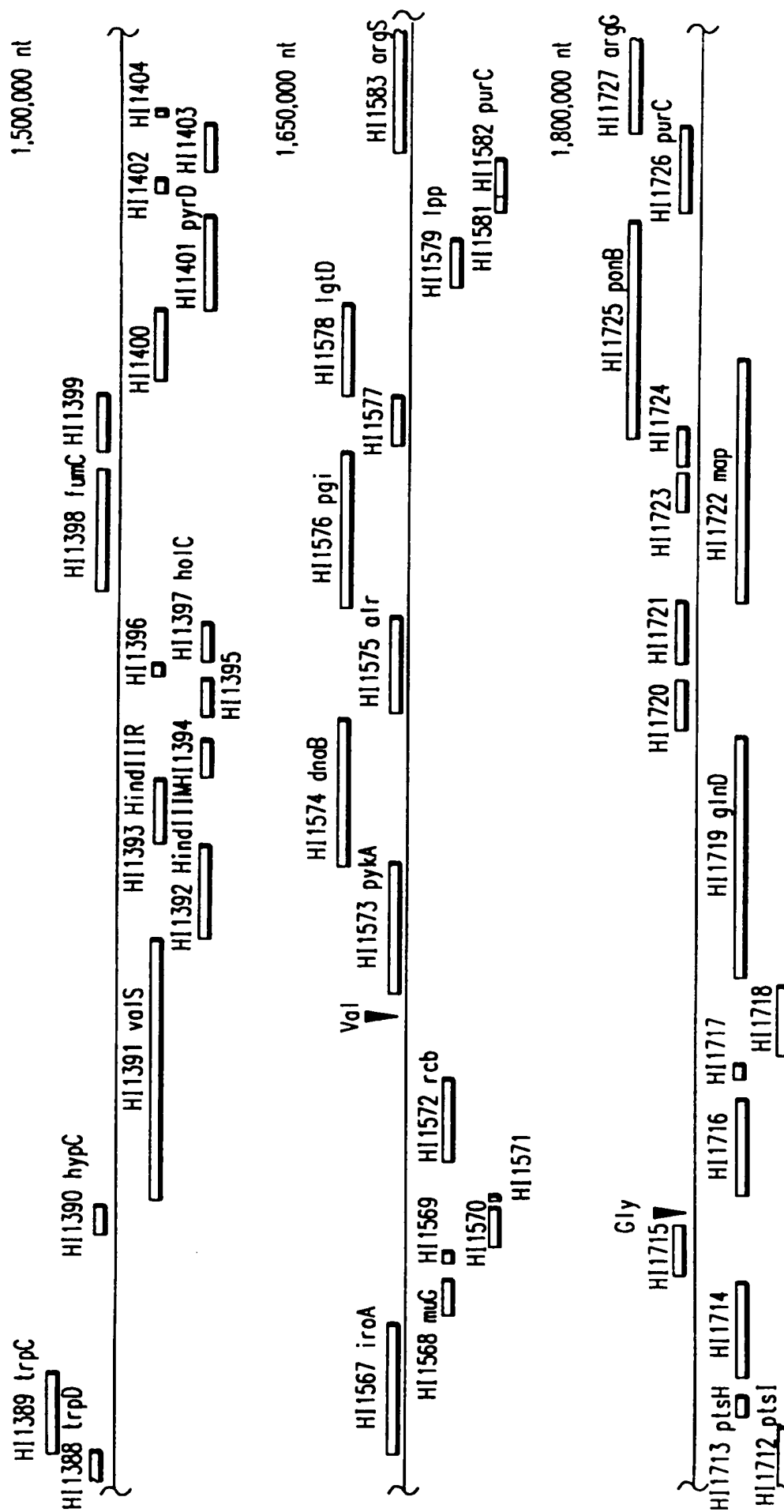
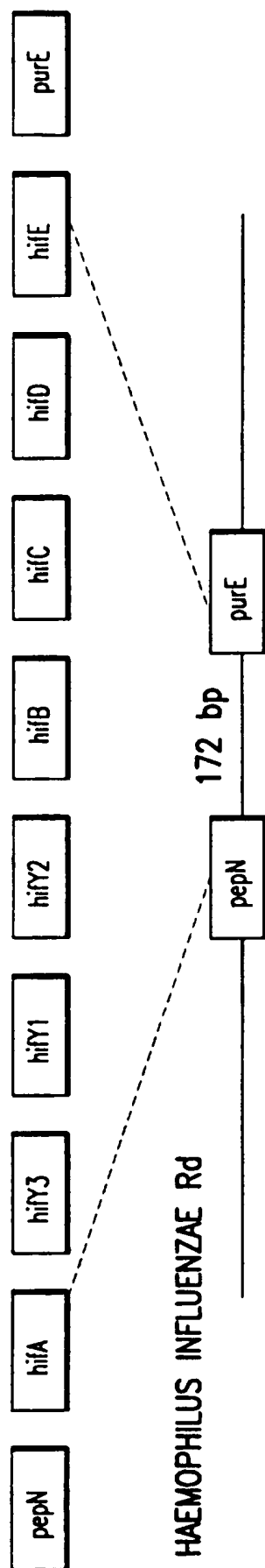


FIG. 6AN

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HAEMOPHILUS INFLUENZAE TYPE b



HAEMOPHILUS INFLUENZAE Rd

FIG.7

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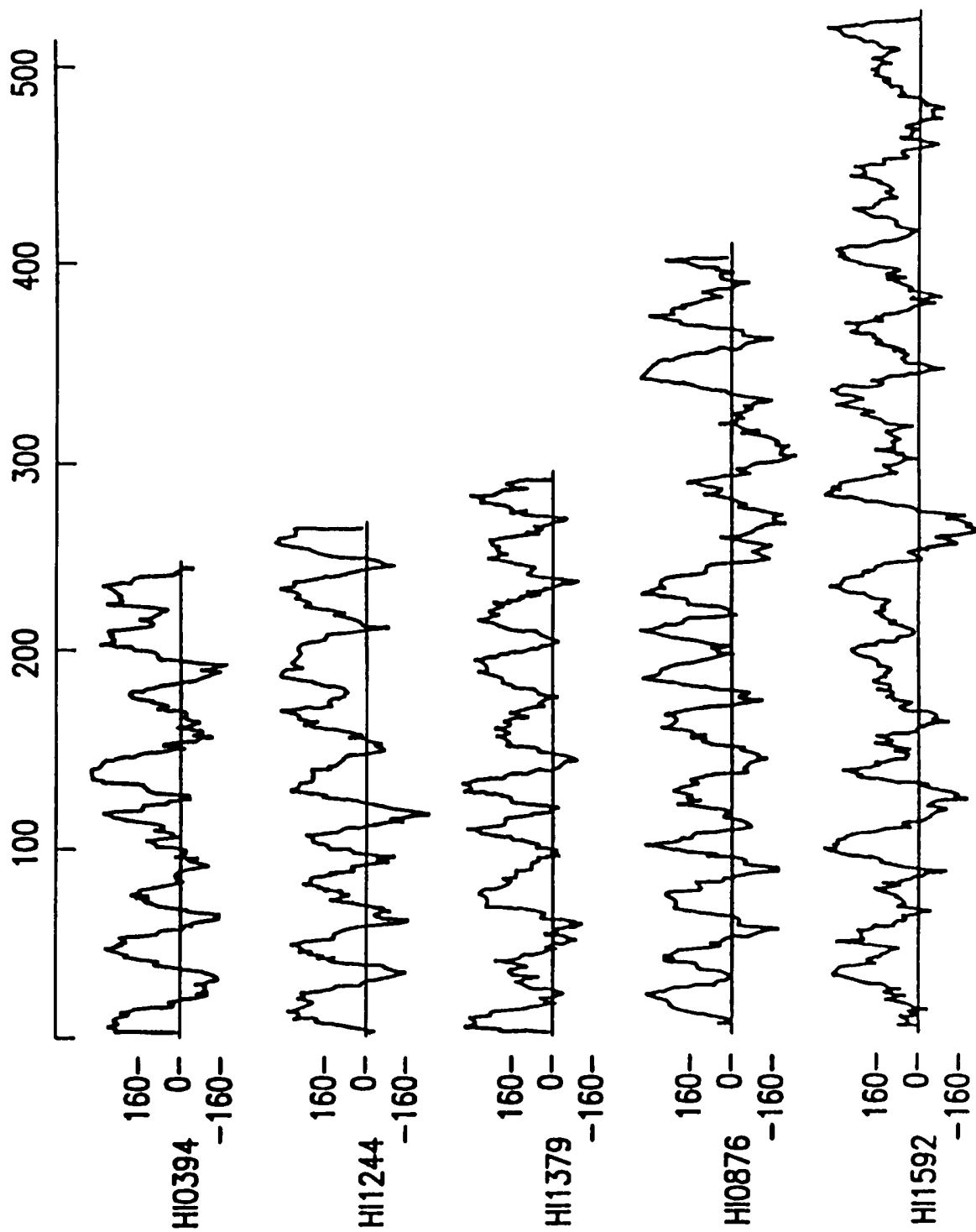


FIG.8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05320

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/31, 15/63, 15/00; C12P 21/02

US CL : 536/23.1, 23.2, 23.7; 435/69.1, 320.1, 172.3, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.2, 23.7; 435/69.1, 320.1, 172.3, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

IGSUITE, FastDB, MPSRCH, nucleotide and amino acid sequence databases searched for elected sequences and open reading frames.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|---|--|
| X --- Y | Nucleotide Database on ENTREZ Release 15.0, published on CD-ROM by National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA, WEISER et al. 'Identification and characterization of oapA, a cell-envelope protein of Haemophilus influenzae contributing to phase variation in colony opacity and nasopharyngeal colonization', nucleotide sequence, 15 February 1995, see entire document. | 18 ----- 8, 9, 11, 12, 15, 16, and 20 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | |
|--|-----|--|
| * Special categories of cited documents: | *T | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *E* earlier document published on or after the international filing date | *Y* | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Z* | document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | | |
| *P* document published prior to the international filing date but later than the priority date claimed | | |

Date of the actual completion of the international search

10 AUGUST 1996

Date of mailing of the international search report

27 AUG 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JAMES MARTINELL

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05320

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-------------------|--|--|
| X | Nucleotide Database on ENTREZ Release 15.0, published on CD-ROM by the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA, COPE et al. 'A gene cluster involved in the utilization of both free heme and heme: Hemopexin by <i>Haemophilus influenzae</i> type B', 15 February 1995, see entire document. | 15 and 16 |
| X,P | COPE et al. A gene cluster involved in the utilization of both free heme and heme: Hemopexin by <i>Haemophilus influenzae</i> type b. Journal of Bacteriology. May 1995, Volume 177, Number 10, pages 2644-2653, see especially page 2648. | 15 and 16 |
| X | SANDERS et al. Identification of a locus involved in the utilization of iron by <i>Haemophilus influenzae</i> . Infection and Immunity. October 1994, Volume 62, Number 10, pages 4515-4525, see especially pages 4520-4521. | 15 and 16 |
| X,P --- Y,P | FLEISCHMANN et al. Whole-genome random sequencing and assembly of <i>Haemophilus influenzae</i> Rd. Science. 28 July 1995, Volume 269, pages 496-512, see entire document. | 8, 9, 11, 12, 15, 16, and 18 ----- 20 |
| Y | WATSON, J. D. et al. "Recombinant DNA in Medicine and Industry". In: Recombinant DNA, Second Edition. New York: Scientific American Books, W.H. Freeman and Company, 1992, pages 453-470, see entire document. | 8, 9, 11, 12, 15, 16, 18, and 20 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05320

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-7
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 1-7 is directed to non-functional descriptive material on computer readable media and is therefore non-statutory subject matter under PCT Rule 39.1.
2. ☒ Claims Nos.: 1, 3-7, 10, 13, 14, and 19
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest

☒

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/05320

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Claims 1 and 3-7 could not be searched in a meaningful manner because the definition of "representative fragment thereof" (page 11 of the description) is such that any sequence found in a database is automatically excluded from being embraced by the claims.

Claims 10, 13, and 14 cannot be searched in a meaningful way because no structural characteristics of the nucleic acids mentioned in the claims are disclosed, hence they cannot be searched.

Claim 19 is directed to antibodies against putative polypeptides. No characteristics of either are disclosed that can be used in a search.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 8, 9, 11, 12, 15, 16, 18, and 20 are directed to no fewer than 961 (Table 1(a) minus Table 1(b)) different DNAs, vectors containing the DNAs, organisms transformed with the DNAs, DNAs encoding homologs of the polypeptides encoded by the no fewer than 961 different DNAs, and methods for producing the polypeptides encoded by the no fewer than 961 different DNAs.

Group II: Claim 17 is drawn to no fewer than 961 different polypeptides encoded by a subset of the encoding DNAs mentioned in Group I.

The polypeptide encoding DNAs, vectors containing them, organisms transformed with them, and methods of polypeptide production using them are materially different from and are therefore independent and distinct from the polypeptides of Group II. Additionally none of the products or methods of Groups I is needed to make the polypeptides of Group II.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Group I contains a separate DNA species for each sequence mentioned. Therefore, there is a minimum of 961 species.

Group II contains at least one polypeptide for each DNA sequence mentioned. Therefore, there is a minimum of 961 species in this Group.

For either Group that applicant elects, a total of 10 (TEN) specified sequences will be searched and no more than 4 (FOUR) specified sequences will be searched for each additional fee paid.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: There is no relationship between or among the various nucleotide and amino acid sequences mentioned in the claims.